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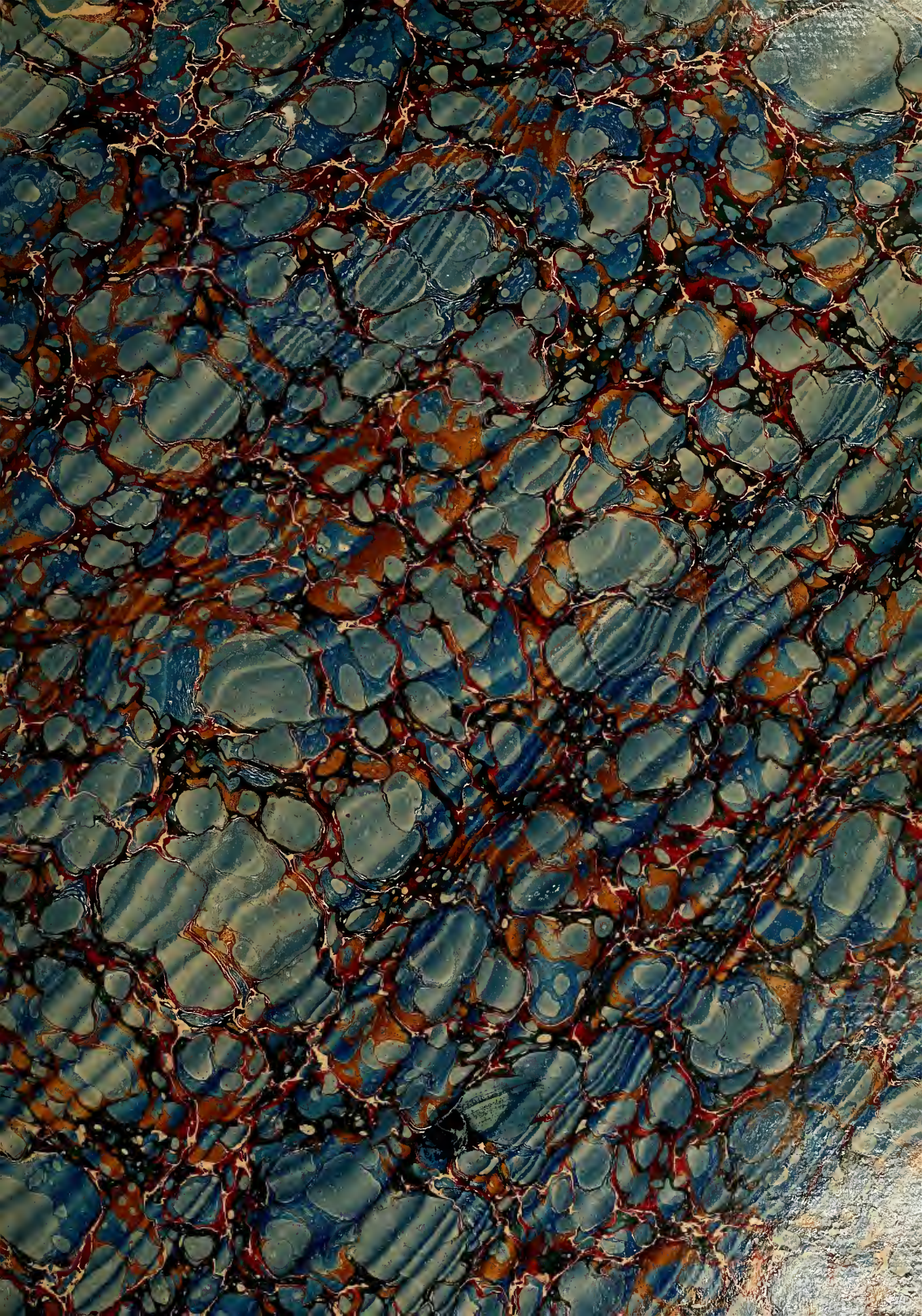

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THE EMBRYOLOGY OF CREPIDULA,

A CONTRIBUTION TO THE CELL LINEAGE AND EARLY DEVELOPMENT OF SOME MARINE GASTEROPODS.

EDWIN GRANT CONKLIN,

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A. INTRODUCTION.

1. *Purpose and History of the Work.*

The purpose of the following work from its inception has been to make as careful a study as possible of the cleavage of the ovum, the formation of the germinal layers and definitive organs, and the axial relations of the ovum to the larval and adult axes. At the time when this work was begun, several years ago, scarcely any attempts had been made to trace the history of individual blastomeres through the entire development to the formation of definitive organs. The early stages of cleavage had received a great deal of attention, but the later stages had been largely neglected; and although the origin and homology of the germ layers was perhaps the most frequently discussed subject in embryology, yet the relation of these layers to the individual blastomeres of the cleaving ovum had been determined in comparatively few cases. Since that

time a number of very valuable papers have appeared on this subject of "cell lineage," as Wilson ('92) has aptly termed it. The results of such work are no longer as novel as they were four or five years ago, and yet the general interest in the subject has greatly increased, and that, too, in spite of the fact that there is a growing school of biologists who believe that individual blastomeres have no necessary relation to future organs. The subject of germ layers is no longer so important as it was once considered; in fact, the theory of the homology of the germinal layers has met with so many difficulties of late that it is now generally maintained only in a greatly modified form. However, the fundamental idea which was prominent in germ-layer discussions is of vital interest to-day. In the whole history of the germ-layer theories I see an attempt to trace homologies back to their earliest beginnings. This problem is as important to-day as it ever was, and whether one find these earliest homologies in layers or regions or blastomeres or the unsegmented ovum itself, the quest is essentially the same.

Within this question of the earliest homologies is included another of great present interest, *viz.*, the significance of cleavage. Is it an orderly sifting of materials, a "mosaic work," or, as Driesch ('93) has maintained in the case of the echinids, a mere quantitative division of homogeneous material? Can the cells of cleaving eggs be compared with each other as the organs of adult animals can? Can one properly speak of the homology of blastomeres? Are the chief axes and regions of the egg or embryo homologous in different animals? And finally, are the causes of the various forms of cleavage to be found primarily in the constitution of the egg itself, in other words, in the internal conditions, or rather in the external conditions, such as pressure, surface tension, gravity, etc.? I know that in these days, when "all the world shakes eggs," it may be hazardous to risk an opinion on these questions which is not based on experimental work. And yet, while fully recognizing the value of experimental embryology, we ought not to forget that "Nature is continually performing some very remarkable experiments in her own way," and I believe we need to know

more about these normal processes before we can properly understand abnormal ones. In order to know the significance of cleavage, it is necessary not only to find out how much the egg may be fragmented or the blastomeres transposed without irreparably destroying development, but also and much more, it is necessary to know every step in the normal formation of the embryo. It is less important to know what remedial processes Nature may have for healing broken eggs, than to understand her usual methods of developing unbroken ones. Whether and how much this "secondary," or regenerative development may differ from the "primary," or normal, is still an open question. If there be a difference, as Roux ('93) maintains, the phenomena of regenerative or secondary development are much more complicated and difficult of explanation than the process of primary or normal development, since in these cases we have to explain the phenomena of normal development plus those of regeneration. In any case the phenomena of normal development are the ones to be explained, whatever method may be used ; and before any explanation can be given it is necessary to know the usual development as thoroughly as possible.

It is because of the perennial interest in these questions of the earliest homologies, and of the significance and causes of the various forms of cleavage, and also with the hope that I may be able either directly or indirectly to add something, however little, to the solution of some of these problems, that I now bring forward this long-delayed contribution on the Embryology of Crepidula.

Crepidula is a genus of prosobranchiate gasteropods, whose development has never heretofore been studied so far as I can learn, — a genus, moreover, which is in many respects a very interesting one, apart from its embryology ; besides, it is so abundant all along our Atlantic coast from Labrador to Florida, and its eggs are so easily obtained, so numerous, and so exceedingly favorable for embryological research, that it seems remarkable that no one has hitherto attempted to study its development.

This work was begun in the summer of 1890, while I was occupying the Johns Hopkins University table at the Marine Laboratory of the United States Fish Commission at Wood's Holl, Mass. During the succeeding winter I continued the work in Professor Brooks' laboratory at Baltimore, and in the summer of 1891 I again occupied the Johns Hopkins table at Wood's Holl, and continued to work on the same subject. Since that time my work has suffered long and repeated interruptions owing to the pressure of other duties.

I had hoped to be able to present in one paper both the earlier and the later stages in the development, but the work has grown so much, both in extent and difficulties, that it has seemed best to publish the results of investigations on the early stages first, and to supplement these by another paper on the later stages as soon as possible. Since the study of the later stages is less general in its bearing and more specifically applicable to the Mollusca, such a division of the subject will not be an illogical nor an unwelcome one. Two preliminary papers have been published on this subject, — one on the general embryology of Crepidula and Urosalpinx (Conklin, '91), the other on the cleavage in Crepidula ('92).

During the first year of the work my attention was directed exclusively to the development of Crepidula fornicata, and a large number of drawings of the various stages in the embryology of this species were made; for this reason it forms the chief subject of this paper, although in some respects C. plana is a more favorable object for study. It was not until the summer of 1892 when, through the courtesy of Professor Whitman, I was enjoying the privileges of the Marine Biological Laboratory at Wood's Holl, that I obtained material for the study of the embryology of C. plana and C. convexa. I have, however, made a careful comparison of the development of these three species, and in most respects have found the cleavage and formation of the germ layers and larval organs very similar in all of them.

Through the kindness of my friend and former pupil, Mr. Harold Heath of the Leland Stanford University, I have recently received a number of adult specimens and a good col-

lection of eggs and embryos of C. adunca, a species quite common on the Pacific coast. I have made a brief study of the embryology of this form. The peculiar features in its development will be referred to later. During the course of this work I have also studied, more or less carefully, the embryology of several other genera of marine prosobranchs, *viz.*, Urosalpinx cinerea, Fulgur carica, Sycotypus canaliculatus, Illyonassa obsoleta, Tritia trivittata, Neverita duplicata.

If space and opportunity permitted, it would be a pleasure to mention the names of many friends who in one way or another have assisted me, but I cannot fail to speak of two or three persons who have placed me under very great obligations. I am indebted to Professor C. O. Whitman, Director of the Marine Biological Laboratory, for the opportunity of working at that excellent institution, as well as for many stimulating suggestions and friendly criticisms ; to Professor W. K. Brooks, my former instructor, for valuable assistance during the first year of my work ; and particularly am I indebted to my wife, who has finished from my camera sketches many of the drawings which illustrate this paper, and has in many other ways rendered me great assistance.

2. *Methods.*[1]

The ova were fixed in many different fluids, — Kleinenberg's picro-sulphuric, picric acid in sea water, Perenyi's, Flemming's stronger and weaker, Merkel's, Auerbach's, Hermann's, corrosive sublimate, chromo-formic, chromo-acetic and absolute alcohol ; but for surface views of the entire egg none of these methods for a moment compares with the first named, *i.e.*, Kleinenberg's stronger picro-sulphuric. The ova were left in this for a length of time varying from fifteen to thirty minutes, and were then gradually transferred to 70 % alcohol. They were left in this until all traces of picric acid had been washed out, and were finally preserved in 95 % alcohol.

[1] The substance of this section was published in the American Naturalist, vol. XXVII (1893).

As a result of many experiments with almost every one of the common staining fluids, I found that the best method of preparing surface views of the whole egg or embryo was the following: (1) Transfer the object gradually from alcohol to water. (2) Stain from five to ten minutes in a solution of Delafield's (Grenacher's) haematoxylin diluted about six times with distilled water and rendered *slightly* acid by a trace of HCl. (3) De-hydrate and clear in oil of cedar or xylol. (4) Mount in balsam, supporting the cover glass so as to prevent crushing. By occasionally softening the balsam with a drop or two of xylol and slightly moving the cover glass the objects can be rolled into any position desired.

By this method wonderfully beautiful surface preparations were obtained, showing with remarkable clearness not only the nuclei and cell boundaries, but also the karyokinetic figures, and in many cases the archoplasmic spheres and centrosomes. One very considerable advantage of this method is that the preparations are permanent — in fact during the first year or two they become better with age instead of degenerating. Most of the preparations from which the figures were drawn are still in existence, and can be consulted at any time.

I have employed this method with almost as good results in the preparation of surface views of the embryo chick and English sparrow, and also with considerable success on other molluscan eggs and embryos, as well as those of annelids and echinoderms.

The objects for sectioning were fixed in various fluids, some of which showed certain points of structure better than others ; for general purposes, however, excellent results were obtained by fixing in the picro-sulphuric solution, though the chromatic filaments and individual chromosomes were brought out much more clearly by the use of absolute alcohol, and the spindle fibres and centrosomes were more clearly shown by the use of Flemming's or Hermann's fluid. In all cases the objects were imbedded in paraffin, and the best results were obtained by staining on the slide. On the whole I have found a double stain, consisting of Delafield's haematoxylin followed by a solution of erythrosine in aniline water, to give the best re-

sults, though many other stains were useful, particularly the Biondi-Erlich mixture and the iron haematoxylin of Heidenhain.

One other thing ought to be mentioned in this connection. I have in no instance been able to follow any one lot of eggs throughout any considerable part of their development. When removed from the mantle cavity of the mother they do not develop normally for more than two or three days. I tried keeping some of the eggs in small dishes, changing the water twice a day; others were placed in a large jar, in which the water was continually aerated by a stream of air; still others were placed in a jar, the mouth of which was covered by silk netting, and the jar was then inverted in a tank of flowing water; the most successful method, however, was to put the eggs in open bottles, which were then placed in an aquarium through which water was constantly flowing. Yet by none of these methods could the eggs be kept normal for more than a few days. It would seem that the circulation of water within the mantle chamber of the mother is more perfect and gentle than could be obtained by any method which I could devise. It was necessary, therefore, to take eggs from a large number of individuals in order to get a complete series, since all the eggs laid by one individual are in nearly the same stage of development. Fortunately, there are such vast numbers of fertile females during the breeding season as to make this an easy task.

B. THE GENUS CREPIDULA.

1. *Natural History.*

At least three species of the genus Crepidula are found on the Atlantic coast of the United States,[1] *viz.*, C. fornicata Lam., C. plana Say, and C. convexa Say, all of which are quite abundant along the shores of New England. All these species are more or less completely sedentary, and they show the most remarkable individual differences in the shape of their shells due

[1] Other species have been described, *viz.*, C. unguiformis Stimson, C. glauca Say, C. acuta Lea. Concerning the first of these there is no doubt that it is identical with C. plana, and I am convinced after a careful anatomical and embryological examination of the last two that they are only local varieties of C. convexa (cf. Verrill '74) Invertebrate Animals of Vineyard Sound.

to the character of the surfaces upon which they are attached. Upon a smooth, plane surface the shell is regular and unusually broad and flat; on a convex surface it is deep and highly arched; on a concave surface it is concave, sometimes to the extent of being almost semicircular; on a twisted surface, like the columella of Neverita, it is twisted; on an irregular surface, such as a rough stone, it is irregular; if pressed upon from the sides the animal and shell become long and narrow; if growth is limited in front the shell becomes short and broad; if limited on all sides the shell may increase greatly in thickness but remains small, filling the space in which it is found. In such cases the lines of growth are crowded closely together and the very edge of the shell may be as thick as any other portion. In small places, such as the interior of Illyonassa shells, C. plana may be dwarfed to one twenty-fifth the size of normal specimens. These individual variations in the shape and size of the animals and shells appear in all the species of Crepidula, but they are most marked in C. plana. The cause of the variations in the *shape* of the shells is not far to seek, though the great differences in the *size* of individuals is more difficult to understand: the shape of the shell is conditioned by the shape and position of the mantle edge; the mantle is moulded over the surface upon which the animal rests; and consequently the shape of the shell comes in time to correspond to any sort of a surface upon which the animal is attached.

C. fornicata, the "slipper limpet" or "boat shell," is a common object to all visitors at the sea-shore. It occurs in great numbers on the shells of the king crab, Limulus polyphemus, where it is firmly attached to the ventral side of the carapace and abdomen; sometimes it is found on the appendages, the gill plates, or even the dorsal surface of the crab. After it has reached a certain size, about half that of the adult, it never moves about. It thenceforth leads a perfectly passive existence, being carried about by the king crab, and obtaining all its food by merely sweeping into its mantle chamber currents of water containing particles of food, which are in large part the crumbs which have fallen from the king crab's table. This species is also found abundantly on muddy sea bottoms a short distance

below low-water mark, where it usually occurs in curious chains often containing ten or twelve individuals. In these chains the foot of one individual is firmly fastened to the dorsal side of the shell of the next one, and the heads of all the animals are turned in the same general direction; the first or oldest individual in a chain is usually attached by its foot to a stone or dead shell. Even those which live upon Limulus sometimes show a tendency to pile one upon another, though in this case there are seldom more than two or three in a pile. C. fornicata also occurs, but in comparatively small numbers, on submerged portions of stones, buoys, and wharves. In none of these cases, however, is it able to change its position after it is about half grown, and it obtains all its food from the particles which float to it in the water. The fact that the large Crepidulas are immovably fixed to one spot is shown not only by the shells, which have in many cases become greatly distorted in order that they may perfectly fit the spot of fixation, but I have again and again observed that in old Crepidulas the sole of the foot secretes a calcareous substance by which the animal is so firmly fixed that the foot is often torn to pieces before it can be freed from its attachments. Unlike most prosobranchs, the foot in Crepidula is plainly divided into two portions, a broad and thin *propodium* which is deeply notched in the middle, and a thick, muscular *mesopodium* or sole, by which the animal is attached. The sole of the foot forms a powerful sucker, and when the animal is removed from its attachment so as not to injure the foot, the latter immediately becomes deeply concave on the ventral side, showing that considerable muscular tension was being exerted in order to produce the suction.

C. plana is smaller and much flatter than C. fornicata, and its shell, which is quite fragile, is nearly white in color. It is found most abundantly within those gasteropod shells (Neverita, Lunatia, etc.) inhabited by the larger hermit crab, Eupagurus Bernhardus, and while it may be found in this position either at the outer or inner lip of the shell, it is nearly always so situated that its head is directed toward the opening of the shell in which the crab lives. It is evident that in this case also the Crepidula has taken this position in order that it may be car-

ried about and supplied with food by the hermit, for here again the Crepidula is unable to move about or change its position in the least after it has reached adult size. When a hermit dies, or leaves one shell for another, the Crepidulas in that shell remain attached for some time, but sooner or later perish without attempting to find another shell. Some doubt has been expressed as to whether C. plana is a true species.[1] It has been held that it belongs to the species fornicata, and that those individuals living inside other shells have been slightly modified by their environment, the shell becoming thinner and flatter. There is no doubt, however, that C. plana is a well-marked species, as is shown by its embryological as well as its anatomical differences from C. fornicata.

A very interesting variety of C. plana is found within those gasteropod shells (Illyonassa, Litorina, etc.) inhabited by the smaller hermit crab, Eupagurus longicarpus. This variety resembles the type in all respects save size, being usually less than one-thirteenth the size of adult female specimens found within the larger shells. That this difference in size is not due merely to age is shown by the fact that the dwarfs are sexually mature, and they show by the shape and character of their shells that they are several years old. Apparently, all the organs are perfectly formed, and differ from those of the larger variety only in size. The ova are of the same size as those laid by the larger form, but are fewer in number. The same thing is true of the cells constituting the other organs of the body, so that it may be said that the difference in size between those two varieties is due to the smaller number of cells of which the body of the dwarf variety is composed, rather than to the smaller size of those cells.

There are many evidences that this dwarf form is not a permanent or persistent variety, but only a physiological one.[2] It, like the typical form of this species, is sedentary, and cannot move about after it has reached a certain size. The shape of the shell and body are modified, so that they fit one particu-

[1] Cf. Gould: The Invertebrata of Massachusetts.

[2] It may be doubted whether the word "variety" should be used in this connection at all. However, for lack of a better term, it is employed in its colloquial meaning rather than in a strictly scientific sense.

lar spot and no other ; therefore, the animal cannot migrate to larger quarters after it has grown to its maximum size in the smaller ones. The eggs, embryos, and larvae of the two varieties cannot be distinguished, and since both live together on the same beach, under about the same conditions of food, temperature, and water, it seems probable that the later development of both would be the same if one was not forced by the smaller size of the shell which it inhabits, or by the smaller quantity of food supplied to it, to remain smaller than the typical form. But what is absolutely conclusive is the fact that the dwarfs, when placed in positions where they can obtain a new foothold and increase in size, become almost, if not quite, as large as the common form. A few specimens were found which showed by the shape and character of their shells that for several years they had lived in the shells inhabited by the smaller hermit crab, and had been typical dwarfs; afterward, having been detached, they by rare good fortune gained a new foothold on a larger surface, and their shells began to increase in size, the new portions of the shell becoming shaped so as to fit the surface upon which they had found a new home. In every such shell one can recognize both the dwarf and the normal forms. The dwarfs are what they are by reason of external conditions, and not because of inheritance. In such a case the *shape* and *size* of the body, and the *number of cells* in the entire organism are greatly modified by the direct action of environment. There is no evidence, however, that these modifications of the shape and size of the body and the number of cells have become in the least degree heritable.

C. convexa is smaller than either of the preceding species, and as its name indicates, its shell is more convex, while its color is much darker than either of the others. It is found upon the *outside* of those gasteropod shells (chiefly Litorina and Illyonassa) inhabited by the smaller hermit crab, Eupagurus longicarpus ; and it undoubtedly obtains its food, as do the others, from the fragments left by its messmate. Unlike the others, however, it can move about to a limited extent, and, if removed from the surface to which it is fastened, can attach

itself again, though so far as I could observe it never voluntarily leaves the shell upon which it is carried about. It is also said[1] to be found in numbers on blades of eel grass, though I have not seen it in such positions.

C. adunca, a species abundant along the Pacific coast, is remarkably like C. convexa in size, shape, and color of shell, as well as in habits and development. Keep, in his *West Coast Shells*, says of it: "The most common species is C. adunca Sby., hooked slipper shell. The apex is strongly recurved, giving the shell a hooked appearance. Its color is brown, but the deck is white. Living specimens may often be found growing upon rocks or upon other shells. Common length from one-half to three-fourths of an inch. Abundant." Mr. Harold Heath, who has been kind enough to send me specimens of this species, together with material for a study of its embryology, writes me that individuals are found in about equal numbers upon the shells of the "black turban" (Chlorostoma funebrale), and upon shells inhabited by hermit crabs. "The individuals found upon the 'black turbans' seem to come to sexual maturity earlier than upon the hermit shells. Several times on pulling off shells of adunca from the 'black turbans,' I was surprised to find eggs under very small shells, very much smaller than are found with eggs on the hermit crab shells." It seems to me that we have here a case parallel with C. plana and its dwarf variety, though the difference between the two forms in C. adunca is very much less striking than in the case of C. plana. That the phenomena in the two species are similar is still further borne out by the fact that the average number of eggs laid by each individual of C. adunca found upon the "black turbans" is 173.3, while the average number laid by those on hermit crab shells is 201.1. Concerning the habits of C. adunca, Mr. Heath writes: "Their shape indicates that they never leave the spot to which they first become attached. Sometimes surrounded by Bryozoa, the shell is clear within the Crepidula shell. Still, when taken off, they can, and sometimes do, regain a foothold. Many that I placed loose in the aquarium have attached themselves to the 'black

[1] Cf. Gould: The Invertebrata of Massachusetts.

turbans' living there. They appear to breed throughout the whole year."

2. *Breeding Habits.*

The breeding season of C. fornicata lasts on the New England coast from early summer until about August 15. A large proportion of the individuals of this species, examined late in June, were found to have laid their eggs, while none were found with eggs later than the middle of August, though many from widely separated localities were examined. At that time, however, the shells from all these localities were covered by the very small young, or spat, of this species. It may be worth while to remark that the breeding season is always earlier with those individuals found on the shells of Limuli than with those which exist in chains on the sea bottom. This is due, I think, to the fact that in early summer the Limuli are found on shallow, sandy beaches, where the temperature of the water is higher than at a depth of one to six fathoms, where the others are found. The breeding season of C. plana begins somewhat later and lasts longer than that of C. fornicata ; several of the former species were found with newly laid eggs as late as September 7. The egg-laying season of C. convexa lasts through nearly the same period as that of C. plana.

As is well known, the sexes are separate in these gasteropods, and the males are fewer in number and smaller than the adult females. Chains of Crepidulas are sometimes found in which there is not a male individual, while isolated females, with from ten to twenty thousand perfectly fertilized eggs, are of frequent occurrence. Considering the sedentary character of these mollusks, the manner of sexual union is an interesting question. There is no doubt that the spermatozoa mingle with the ova before the egg capsules are formed within the oviduct of the female, and yet the mature females are absolutely fixed to one spot, and the largest males have very little, if any, power of movement. The smaller the individual is, however, the greater its power of locomotion. The young of both sexes are freely motile, but as they grow larger they lose this power. In C. plana all the males are much smaller than

the females, and all are motile. In C. fornicata the males may become almost as large as the females, in which case they become immovably fixed to one spot, and cannot, therefore, perform the sexual function unless they are attached near to or upon a female. In C. convexa and in C. adunca all the males are smaller than the females, and are motile. I have carefully taken the volume of a number of alcoholic specimens, and find that the following ratios exist between the males and females of the different species: in C. plana the males are about one-sixteenth the size of the females; in C. adunca, one-eighth; in C. convexa, one-fifth; in C. fornicata, three-quarters. The small males are able to move about more or less freely; if they are detached they readily find a new foothold, and their shells are rarely distorted to fit irregular surfaces, as is the case with the females. There is, then, a marked sexual dimorphism in these mollusks, the mature females being generally much larger than the males; the females are sedentary, the males locomotive, and at the breeding season, or perhaps once for all, the females are visited and fertilized by these motile males. In all mature females, the seminal receptacle, which is a convoluted tubule communicating with the oviduct, is at all times filled with mature spermatozoa. These spermatozoa are attached to the walls of the receptacle by their apices, while their tails project into the lumen exactly as they do in the seminiferous tubules of the male. I believe that the spermatozoa receive nutriment from the walls of the seminal receptacle, and that they can live in this position indefinitely. Since there are myriads of spermatozoa in the receptacle, and furthermore, since none are wasted, so far as I have been able to observe, it might well be that copulation occurs only once in a lifetime.

In C. plana the shell of the male has a characteristic shape, being more nearly round than that of the female, and having a rather sharply pointed apex. This shape is so characteristic that it is generally easy to distinguish a male from an immature female. I have observed a good many cases in which the older part of the shell had the male characters while the newer part was like that of the female. In such animals the penis is

usually very small, and in some cases has almost entirely disappeared. Quite a complete series of stages in the degeneration of this organ was observed, from the fully formed organ on the one hand to a minute papilla on the other. Sections of such animals show that neither male nor female sexual cells are produced at this time. Although the evidence seems to favor the view that we have in these cases an example of successive hermaphroditism, I am not able to assert that this is really the case, although I have spent considerable time in attempting to decide it.

3. *Types of Development in C. fornicata, C. plana, C. convexa, and C. adunca.*

All the ova produced by one individual are laid at about the same time, and the development proceeds very slowly. In C. plana and C. fornicata it is about four weeks from the time the ova are laid until the fully formed veligers escape from the egg capsules, and in C. convexa and C. adunca the period preceding the escape of the young is probably much longer. How long the veligers of the two former species lead a free-swimming life I do not know, since I found it impossible to keep them alive until they were transformed into the spat, or young Crepidulas. From circumstantial evidence, however, I am convinced that in C. fornicata the veligers do not swim about for more than three weeks, probably about two. On July 23, 1890, Mr. Vinal Edwards, collector for the United States Fish Commission, brought me a large number of C. fornicata, dredged from the mouth of the New Bedford river. A large proportion of these were carrying egg capsules, many of which contained fully formed veligers, while most of them were in an advanced stage of development. On August 11, nineteen days later, another lot of Crepidulas were taken at the same place, but no eggs or egg capsules could be found; the parent shells, however, were covered with the very small spat of this species. During July of the next year (1891) I kept a lot of veligers of this species in a wooden box, the bottom of which was covered by silk netting. The box was

anchored in the "codfish pool," a place where there was a large supply of fresh and pure sea-water, and yet where the surface was generally calm. In this box some of the veligers lived for almost two weeks, but although there were stones and shells in the box, I could not find any spat upon them at the end of that time. From these facts it seems probable that the free-swimming life of the veligers lasts not less than two weeks nor more than three. The whole course of development, therefore, from the time the eggs are laid to the close of the larval life and the assumption of adult characters and habits, is from six to eight weeks.

The fertilized eggs in all four species are laid in capsules, which are formed by secretions from the wall of the uterus or nidimental organ.[1] These capsules are united into a bunch, like a cluster of grapes, by a common stem, which is fastened to the shell, stone, or other object upon which the Crepidula lives. This bunch is attached between the two folds of the propodium, and within the mantle cavity of the mother, and since the adults do not move about, it follows that the eggs are always covered by the parent's shell. As a result of this protection the walls of the egg capsules are thin and delicate; very unlike the tough, leathery capsules of most marine prosobranchs. Within the capsules is an albuminous fluid, in which the eggs are immersed, and which is absorbed by the embryos in the course of development. Salensky ('72) has described similar capsules and egg-laying habits in Calyptraea, a sedentary prosobranch nearly related to Crepidula.

The approximate number of capsules and eggs deposited by the mature females of the different species is shown in the following table:

[1] The capsules in Urosalpinx cinerea are marked on the outside by faint spiral lines, and show a tendency to tear in a spiral direction. The same is true of the capsules of Crepidula, though in a less marked degree than in Urosalpinx. This spiral structure is caused, I think, by the rotation of the capsule as it passes through the uterus, in the same way that the spiral character of the egg membranes of birds and reptiles is produced.

Table I.

	Number of Capsules.	Eggs in Each Capsule.	Total Number of Eggs Laid.
C. fornicata,	55	240[1]	13,200
C. plana (type),	51	176	9,000
C. plana (dwarf),	48	64	3,070
C. convexa,	20	11	220
C. adunca,	10	18	180

These figures are but rough averages made from counting the capsules and the eggs in many of the capsules laid by a large number of mature females; I have no doubt that in another lot of individuals the numbers would be found to vary a little from those given above. In general, however, these figures may be taken as approximately accurate. In all cases the smaller the individual of a species the smaller the number of eggs laid, so that two specimens scarcely ever lay the same number of eggs.[2]

This great difference in the number of eggs laid is the result of the different modes of development in the different species. In no species which is not rapidly increasing or decreasing in numbers are more or less ova produced and fertilized than are just sufficient to insure the continuance of that species in its present numbers. There is no reason to believe that any of these species of Crepidula are rapidly increasing or decreasing in numbers at present; so far as one can judge, each is just about holding its own. If, therefore, one species produces sixty or seventy times as many eggs as another, it must be that in the one case each fertilized ovum has sixty or seventy times as many chances of reaching maturity as in the other case. The history of the development

[1] By a typographical error in a former paper ('92) it is recorded that "about 50 eggs are laid in each pouch or capsule" of C. fornicata. It should read "about 250."

[2] Herrick ('91) showed that the number of eggs laid by the American lobster varies greatly, depending upon the size of the lobster. More recently ('95) he has published an extensive series of measurements of female lobsters and computations of the number of eggs laid by them, from which he constructs the curve of the fecundity of the lobster. He concludes that "the number of eggs produced by female lobsters at each reproductive period varies in a geometrical series, while the lengths of the lobsters producing these eggs vary in an arithmetical series."

in each of these species shows that this is probably the case, for associated with these differences in the number of ova produced are profound differences in the later stages of development. C. plana and C. fornicata pass through a long larval or veliger period, but C. convexa and C. adunca have no free-swimming larval stage at all, the young crawling directly out of the egg capsules in a condition practically adult. Since vast numbers of the free-swimming veligers of C. plana and C. fornicata must be destroyed before reaching that stage of development at which the young of C. convexa and C. adunca first issue from the egg capsules, it is evident that vastly more eggs must be produced by the two former species than by the latter if these different species are to continue in the same relative numbers in which they are now found.

Correlated with the different number of ova produced by the three species are noteworthy differences in the size of the ova, as is shown by the following tables and diagram :

TABLE II.

ABSOLUTE MEASUREMENTS OF THE UNSEGMENTED EGGS OF CREPIDULA. (APPROXIMATE.)

(All measurements were made on eggs preserved in alcohol and mounted in Canada balsam.)

SPECIES.	DIAMETER.	VOLUME.	NUMBER OF EGGS LAID.
C. plana (type),	.136 mm.	.00131709 cu. mm.	9000
C. plana (dwarf),	.136 "	.00131709 " "	3070
C. fornicata,	.182 "	.00315655 " "	13200
C. convexa,	.280 "	.01149406 " "	220
C. adunca,	.410 "	.03608703 " "	180

TABLE III.

RELATIVE MEASUREMENTS OF THE UNSEGMENTED EGGS OF CREPIDULA. (APPROXIMATE.)

SPECIES.	DIAMETER.	VOLUME.	NUMBER OF EGGS LAID.
C. plana (type),	1	1	50
C. plana (dwarf),	1	1	17
C. fornicata,	$1\frac{1}{3}$	$2\frac{2}{5}$	73
C. convexa,	2	$8\frac{3}{4}$	$1\frac{1}{3}$
C. adunca,	3	$27\frac{2}{5}$	1

This dissimilarity in the size of the eggs is due to the differences in the larval history — the species with the most pronounced larval period having the smallest eggs, because but a small amount of nutritive yolk is necessary to carry the development to the free-swimming stage where the larva can take care of itself, while in the species without any larval history enough yolk must be stored in the egg to carry the development clear through to the adult condition.

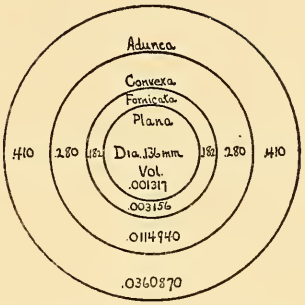


DIAGRAM 1. — Showing the relative size of the eggs of C. plana, C. fornicata, C. convexa, and C. adunca. The actual diameter and volume of each is given in millimeters and cubic millimeters.

The larger size of the eggs in C. adunca and C. convexa as compared with C. fornicata and C. plana, is due chiefly to the greater amount of yolk stored in the entoderm cells of the two former species; and it is worthy of note that this increased quantity of yolk is equally distributed, so that the four macromeres produced by the first two cleavages are nearly equal in size and bear the same relation to each other in the larger eggs that they do in the smaller ones, though in many other molluscan eggs, *e.g.*, Aplysia, Urosalpinx, Unio, and Ostrea, one of the macromeres is very much larger than the other three.

In spite of this vast difference in the size of the eggs in these different species of Crepidula the cleavage, gastrulation, and formation of organs is very similar in all of them. In the large eggs of C. adunca and C. convexa the entoderm cells are, relative to the ectoderm cells, much larger than in C. fornicata, and C. plana; therefore, at the time of the closure of the blastopore there are more ectoderm cells in the large eggs than in the small ones. A count of the nuclei of the ectoderm cells in the species plana, fornicata, and convexa at this stage, shows that they are to each other as two, three, and five respectively, while a comparison of adunca with the other species at an earlier stage (just before the division of the three smaller entoderm cells, see p. 156), shows that it has a larger number of ectoderm cells than either of the others.

The cleavages are precisely the same in all the species up to the 52-cell stage. At this point the ectoderm cells begin to grow more numerous in adunca, though the divisions continue the same until a still later period in the other species.

In all the species the number of mesoderm and entoderm cells remains the same as far as they can be recognized.

There can be no doubt that C. plana and C. fornicata, with their larval types of development, represent a more ancestral condition than C. convexa and C. adunca with their suppressed larval or foetal type.[1] It must be considered that the larval type of development is the more ancestral, from which the foetal type has been derived. The small number of eggs and the direct development of C. convexa and C. adunca are correlated with the small size of the adult in these species, and this in turn may be due to the action of environment through natural selection. These species live upon small objects, chiefly those small gasteropod shells like Litorina or Chlorostoma, which are inhabited by the small hermit crab, and only those individuals could survive in these positions which are small enough to become firmly attached to these shells, while all larger ones would be torn off, and would sooner or later perish. The dwarf variety of C. plana furnishes evidence that the cause here assigned for the small size of C. convexa and C. adunca is not purely imaginary. The ability which all the members of this genus show to adapt themselves to large or small places, and to modify the shell so that it will fit plane, convex, concave or angular surfaces indicates, that the body is very plastic.

But whatever the cause of the smaller size of C. convexa and C. adunca may be, it is evident that the total mass of germinal matter must be less in these than in the larger species, provided that all the other organs are developed in about the same relative proportions, as appears to be the case.

[1] The use of the expression "foetal type of development" in this case is, I think, justifiable. It is true that in these four species various stages in the suppression of the larval development are shown, and even in that species in which the larval development is most completely suppressed, *viz.*, C. adunca, there are many rudiments of larval organs; yet these are only rudiments and they completely disappear before the young escape from the capsules.

Clearly two methods of reducing the total amount of germinal matter are possible : (*a*) the germ cells, while remaining the same in number, may decrease in size, or (*b*) the germ cells may decrease in number, provided a larger proportion of them produce adults. Both of these methods are illustrated within the genus Crepidula. (*a*) The typical form of C. plana, which is about one-third the size of the average C. fornicata, produces almost as many eggs as the latter, but each egg is only about one-third the size of the eggs of C. fornicata. In this case the total amount of germinal matter has been decreased (or increased, according as one or the other species is taken as a standard) by the decrease in *size* of the individual cells. (*b*) In the dwarf variety of C. plana, which is only one-thirteenth the size of the type form, the eggs are of the same size as in the common variety, but much *less numerous*. The method of development in the two varieties is exactly the same, and therefore it follows that unless the typical variety is rapidly increasing in numbers, which does not appear to be true, the dwarf variety must be rapidly disappearing. I think it altogether probable that the eggs laid by the dwarfs are not numerous enough to continue the dwarf variety in its present numbers, and it would rapidly disappear if it were a true or morphological variety. However, since it is merely a physiological variety of C. plana, due to the smaller size of the shell in which the young take up their residence, the continuance of the dwarf variety is not dependent upon the number of eggs produced by the dwarfs ; rather it depends upon the number of the young of C. plana, whether of the common or dwarfed form, which make their abode in the smaller shells.

In C. convexa and C. adunca the amount of germinal matter is reduced in the same way that it is in the dwarfed form of C. plana, *i.e.*, by reducing the number of cells. Since, however, these are true species which are neither rapidly increasing nor decreasing in numbers, it follows that if they produce a smaller number of eggs than the other species, the chances that each egg will produce an adult must be proportionately increased. In C. convexa and C. adunca this is done simply by lengthening the period during which the young organism

remains under the protection of the mother, *i.e.*, by the suppression of the larval type of development.

Since the yolk is almost the only nutriment furnished the young organisms by the mother, it follows that the sooner they can begin to take care of themselves the less yolk will be needed, while the longer they remain in the egg capsules the more yolk will be required. While it is true, therefore, that in the foetal type of development the number of germ cells may be decreased, it is also true that the size of each ovum must be increased. However, in C. convexa and C. adunca the decrease in the number of eggs more than overbalances their increase in volume, so that the total volume of eggs laid is greatly reduced as compared with the other species. The following table gives a basis for comparing the approximate volume of the body of a mature female in each species with the total volume of the eggs laid:

TABLE IV.

COMPARISON OF VOLUME OF ADULT WITH VOLUME OF EGGS LAID.

SPECIES.	RELATIVE NO. OF EGGS.	RELATIVE VOL. OF SINGLE EGG.	RELATIVE TOTAL VOL. OF EGGS LAID.	RELATIVE VOL. OF ADULT FEMALE.
C. convexa,	$1\frac{1}{3}$	$8\frac{3}{4}$	1	$1\frac{1}{4}$
C. plana (dwarf),	17	1	$1\frac{1}{2}$	1
C. adunca,	1	$27\frac{2}{5}$	$2\frac{1}{3}$	$4\frac{1}{6}$
C. plana (type),	50	1	$4\frac{1}{4}$	$13\frac{1}{3}$
C. fornicata,	73	$2\frac{2}{5}$	15	30

The first series of measurements which I made showed a close correspondence between the relative total volume of eggs laid and the relative volume of the adult in these different species. Later and more careful measurements have given the results set down in the above table. The fact is that the sexually mature females of a species vary so much in size, and the eggs laid by them vary so greatly in number, that unless one measures a very great number of individuals of all sizes, no satisfactory ratio between the eggs laid and the volume of the adult can be determined for a given species. However, all measurements and enumerations show that the volume of eggs

laid is, in general, directly proportional to the volume of the adult. This is very plainly the case within a single species where the *number* of eggs laid always stands in direct relation to the size of the animal which lays them. When one species is compared with another this same thing is generally true of the total *volume* of eggs laid, *i.e.*, the species with the largest individuals lays the largest volume of eggs, though the number and size of the eggs in the various species differs immensely. For example, C. convexa, which is about one-twentieth the size of C. fornicata, produces only about one-sixtieth as many eggs of one-fifteenth the total volume of those laid by the latter species. The great reduction in the number and total volume of eggs in C. convexa and C. adunca, as compared with the other species, is made possible by their foetal type of development. At the same time the wide distribution of individuals brought about by the free-swimming veligers of C. fornicata and C. plana is partially secured in C. convexa (I do not know whether this is true of C. adunca or not) by the freely moving young or spat of this species, which are much more active than the spat of either of the other species.

It is very evident that the foetal type of development in C. convexa and C. adunca is correlated with the smaller size of the adult in these species, and for the reasons given above, it seems to me probable that the former may be in some way the *result* of the latter.[1]

4. *General Sketch of the Embryology.*

The First and Second Cleavages. — The chief axis of the ovum corresponds to the future dorso-ventral axis of the embryo.

[1] Although I do not suppose that these relations between the size of the adult and the number, size, and volume of the eggs produced, is a general law applicable to all larval and foetal types of development, neither do I think that such relations are wholly isolated, *i.e.*, true only of this one genus, Crepidula. I believe they will be found to be quite generally true of the gasteropods. Long ago, Fol ('**76**) called attention to the fact that among the heteropods the smallest species lay the largest eggs. He says, "The smallest heteropods lay relatively the largest eggs, but infinitely fewer than the larger species." He did not observe that the largest eggs had a foetal or suppressed larval development, but I think it would be safe to assume that this is true, and that here also the foetal type, and consequently the larger eggs, are due in part to the smaller size of the adult.

The first cleavage is transverse to the long axis of the embryo, exactly as it is in the case of Teredo, Nereis, and Umbrella, and divides the ovum into an anterior and a posterior half; the second cleavage coincides with the antero-posterior axis of the future embryo, and divides the ovum into right and left moieties. The four macromeres formed by the first two cleavages are nearly equal in size, and each contains the elements of both ectoblast and entoblast, and the left posterior macromere contains, in addition, most of the future mesoblast.

Formation of the Ectoblast. — The whole of the ectoblast is separated from the macromeres by three successive divisions, which separate twelve micromeres from the four macromeres. The four cells first separated from the macromeres constitute the first quartette of micromeres, while those separated by the two following divisions are respectively the second and third quartettes. The first quartette forms the upper hemisphere (umbrella or head vesicle) of the larva, the brain, an apical sense organ, an apical plate of ciliated cells, and a portion of the velum. The second quartette gives rise to the larger part of the velum, the shell gland, and at least a part of the foot. The third quartette I have not been able to follow satisfactorily; its derivatives lie wholly outside of the velar area, and form a considerable part of the lower hemisphere.

Formation of Mesoblast and Entoblast. — Soon after the ectoblast has been segregated, and at the stage when there are twenty-four cells, the left posterior macromere divides obliquely, forming the first member of the fourth quartette, which later comes to lie in the second cleavage furrow at the posterior side of the egg. This cell then divides into right and left portions, and each half again divides into a dorsal and ventral part. The two ventral moieties form a part of the intestine or hinder portion of the alimentary canal. The two dorsal moieties are still mesentoblasts, and the mesoblast is not completely separated from the entoblast until after two more divisions. There are finally formed two mesoblastic teloblasts, each of which gives rise to a mesoblastic band, from which a part of the middle layer is derived. The rest of the middle layer comes apparently from one additional mesoblast cell in

each quadrant, except the left posterior one. These three cells are derived from the advancing edge of ectoblast, and from them the scattered mesoblast cells around the blastopore apparently originate. The other three members of the fourth quartette are purely entoblastic, and they form the lateral and ventral walls of the mesenteron. The residue of the four macromeres is entirely entoblastic, and after they have given rise to a fifth quartette of large yolk cells they form the dorsal wall of the mesenteron.

Gastrulation. — The gastrula is formed by epibole associated with a flattening of the macromeres ; there is no invagination. The blastopore closes near the middle of the ventral side, and at this point the mouth soon afterward appears.

The Ectoblastic Cross. — When the stage with forty-two cells has been reached, there appears at the upper pole of the egg a cross of ectoblast cells ; the centre of the cross lies exactly at the animal pole, while each of the arms lies between the first and second cleavage planes. Later the whole cap of ectoblast shifts position so that the arms of the cross lie approximately over those cleavage furrows ; thus one arm comes to be anterior, one posterior, one right, and one left. In the further development all the arms lengthen, and all save the posterior one divide longitudinally into two parallel rows of cells. All the cells of the cross are derived from the first quartette save the "tip," or terminal cell, of each arm, which comes from the second quartette. A single ectoblast cell, which is at one time the smallest in the egg, but which afterwards becomes the largest, lies in the angle between adjacent arms of the cross. There is one of these in each quadrant, and because of their position and shape they are called for the present the "turret cells." In later stages at least two of them contribute to the formation of the velum.

Change of Axes. — During the later stages of cleavage and throughout gastrulation, the whole of the ectoblast at the upper pole moves gradually forward through an angle of about 90°, so that the centre of the cross, which originally lay at the middle of the future dorsal region, comes to lie at the anterior end of the long axis of the embryo. The entoblast seems to

take no part in this shifting, and the ectoblast on the postero-ventral side of the ovum moves in an opposite direction, *i.e.*, forward on the ventral side. There is thus a stationary point in the ectoblast on the posterior side of the egg, in front of which the ectoblast cells are shoved forward, both on the dorsal and ventral sides. This stationary point is just ventral to the region of the future shell gland, and probably corresponds to the posterior growing-point of the annelids.

Organs formed from First Quartette. — Those cells of the first quartette which lie posterior to the lateral arms of the cross, grow very large and become covered by fine cilia, which protrude through a thin cuticula. These are the cells of the posterior cell plate, and they form the principal part of the walls of a large head vesicle.

The four central or apical cells give rise to an apical sense organ. Each cerebral ganglion is formed at least in part from the cells of the "rosette series" lying on each side of the mid line and between the bases of the anterior and lateral arms of the cross; secondarily the ganglia become connected with the apical organ and with the pedal ganglia and otocysts. The eyes are formed in connection with the cerebral ganglia. All the turret cells lie in the velum, and at least the two anterior ones contribute to the formation of the first velar row. The cells of the lateral arms of the cross divide repeatedly, and some of them form part of the velum. The anterior arm forms a plate of seven large cells reaching from the apical cells to the velum.

Organs formed from the Second and Third Quartettes. — A portion of the velum completely surrounds the first quartette. That part of the first velar row which lies at the ends of the arms of the cross, is formed from the second quartette; the intervening portions, on the anterior side, come from the first quartette (turret cells). The velum is many cell-rows wide, and consists of a preoral and postoral ridge bearing long flagellae and an adoral ciliated groove lying between the two. Dorsally the velum divides into anterior and posterior branches, which are separated by the posterior turrets and the other cells of the posterior plate. The anterior branch runs in on each side toward the apex, and ends on each side of the apical organ;

it traverses the cells of the transverse arms from tip to base. The posterior branch, which is never functional, surrounds the first quartette. When first formed the velar cells are not ciliated, and they lie at the same level as the surrounding cells. Later they are raised into a well-marked ridge, and are finally drawn out into a very extensive wheel-shaped lobe, the long velar cilia being borne around its margin.

The shell gland appears on the postero-dorsal surface just dorsal to the growing-point. It arises as a prominence of ectoderm cells, which from their position seem to be derived from the posterior member of the second quartette. In the place of this prominence an invagination afterward appears ; the margins of the invagination extend rapidly, and a thin cuticle, the first indication of the shell, is secreted by the invaginated cells. As development proceeds the shell becomes asymmetrical, developing more rapidly on the left side than on the right. This asymmetry extends to all the organs posterior to the foot and head vesicle.

The foot arises as a single median protuberance on the ventral side of the body just posterior to the mouth, and in front of the anal or growing region. In later stages the foot becomes more and more prominent posteriorly, until it turns forward and lies ventral to the mouth, though still attached to the body posterior to the mouth.

At the posterior end of the embryo three or four large ciliated anal cells appear very near the growing-point, and at this place the distal end of the intestine is in contact with the ectoderm. The proctodeal invagination does not occur until late in development.

Later Changes. — The intestine is a tube with a distinct lumen, its walls being formed of small cells free from yolk. Its posterior end is formed first, and it grows in length chiefly by the addition of cells at its anterior end, where it opens into the space between the yolk cells. In the course of development, the distal end of the intestine is carried forward on the ventral side, and at the same time the whole hinder portion of the embryo undergoes laeotropic torsion. By the continuance of these two movements the distal end comes to lie in front of the central end, and the latter is found successively on the right

side, the dorsum, and the left side of the embryo. In the end the course of the mesenteron is like a figure 8 open at the top.

In well-advanced embryos the head vesicle and the velar folds become separated by a deep constriction from the posterior part of the embryo. The latter contains all the yolk, and it alone becomes asymmetrical ; the head vesicle, velar lobes, and foot, all of which lie anterior to this constriction, retain their bilateral symmetry.

At the point of constriction there is a large spherical prominence on each side, just dorsal to the foot ; this is the primitive excretory organ ("urniere").

On the right side of the embryo, just posterior to this constriction, a depression appears in the ectoderm which becomes the branchial cavity.

The formation of the gills, permanent kidney, pericardium, and heart does not occur until a later period than is shown in the figures.

In later stages the head vesicle decreases rapidly in size, the velum is largely, if not entirely, absorbed, the foot becomes relatively very large, and the shell, which during the veliger stage was of the spiral type, takes on the form characteristic of the adult.

In this condition the young or spat resemble the adult forms in all essential respects, and the embryology may be considered as finished.

5. *Abnormalities.*

Under entirely normal conditions all the eggs of C. fornicata and C. plana develop into perfect embryos and veligers (I have not studied C. convexa and C. adunca with reference to this point) ; still it is not uncommon to find one or two small, abnormal embryos in each egg capsule, even though taken from an individual living in what seems to be a normal environment. But when the adult Crepidulas are removed to the laboratory, and kept in the best possible conditions, the percentage of these abnormalities increases, and when the egg capsules are removed from the mantle cavity of the mother, and kept in dishes of sea-water, the monstrosities increase to such an extent that after a few days not a single normally developing egg or embryo can be found.

These abnormalities may appear in the early stages of cleavage, or they may be found in any of the later stages of development, even up to the fully formed veliger. When present in the early stages the blastomeres are more spherical and less compact than usual. The four macromeres are frequently separated from each other far enough to leave a cavity between them, and into the depression thus formed the overlying ectoderm cells dip down, forming a pit. A similar invagination has been described by Blochmann ('81) for Neritina and by McMurrich ('86) for Fulgur. Both of these authors supposed that this was a normal feature of development, but from the loose character of the cell aggregate and the rounded outlines of each of the cells in Blochmann's figures of Neritina, I believe that the ova there described were segmenting abnormally, and this view is rendered all the more probable when the large proportion (eighty to one) of abnormal as compared with normal eggs in Neritina is taken into account. In fact, Figs. 52–56 of Blochmann's paper represent very well the abnormally segmenting eggs of Crepidula, and I believe the ectodermal pit is in Neritina, as in Crepidula, an abnormal formation.

I have studied the cleavage in Fulgur and find that the ectodermal invagination which McMurrich describes is the shell gland which appears at an early stage, some distance posterior to the apical pole ; it is therefore a wholly different feature from the invagination in Neritina, which lies exactly at the apical pole.

Heymons ('93) found that in Umbrella, one of the Opisthobranchiata, the egg capsules contain thirty to forty eggs, and that some of these, though evidently no definite number, do not develop normally. He says : "Von letzeren kommen nicht alle zur normal Entwicklung, indem ein Theil von ihnen gleich nach der ersten Stadien abweichende Verhältnisse und Missbildungen zeigt und später dem Zerfalle unterliegt. Nicht selten sind auch Doppel- oder gar Mehrfachbildungen zu beobachten, die durch Aneinanderwachsen der Eier zu Stande kommen, wie sich im zwei- oder vierzelligen Stadium leicht nachweisen lässt, und die demgemäss auch die doppelte resp. mehrfache Grösse besitzen. Solche Doppelbildungen sind häufig

noch ziemlich spät, noch nach Anlage des Fusses und der Schalendrüse anzutreffen und können bis auf die Berührungsstelle ganz normal ausgebildet sein."

Such double or multiple formations sometimes occur in Crepidula and many other prosobranchs, though so far as I have observed they never reach so advanced a stage as Heymons mentions. Among the later stages in Crepidula the abnormal forms are sometimes nearly like the normal ones, the chief difference being due to irregularities of form, which often take the shape of protrusions or wart-like prominences. A more marked form of degeneration is shown by those embryos which have divided into two or more pieces, each of which may move about independently. It is not an uncommon thing to see a little embryo consisting largely of velum and foot, and entirely disengaged from the yolk cells, swimming actively about in the most amusing fashion. Still greater degrees of degeneration are shown by small fragments, which move about rapidly and are nothing more than little masses of ciliated cells.

It might be considered that in all such cases these abnormal forms were the result of unfavorable conditions, such as imperfect aeration, varying density of the sea-water, or rough mechanical treatment, were it not for the fact that in some forms (*e.g.*, Neritina) even under the most perfectly normal conditions a definite number of abnormalities are always found. McMurrich ('86) has given a pretty complete series of forms showing the varying tendency to produce abnormalities which different species possess. In Fulgur and Urosalpinx all the eggs are said to develop ; in Purpurea floridana all do not develop, but a considerable number (not definitely stated) break down and are used as food : in Purpurea lapillus there are five hundred to six hundred eggs in a capsule, only twelve to thirty of which develop, while in Neritina fluviatilis there are seventy to ninety eggs in each capsule, only one of which undergoes regular development. In each of these cases the eggs which do not develop, break down and are used as food by the normal embryos. Such cases cannot be accounted for by assuming merely that the environment is unfavorable. Such a cause would give no such definite results as are said to exist, *e.g.*, in

Neritina. Nor can it in all cases be explained by assuming that in each egg capsule there is a struggle for existence, and that the fittest survive while those less hardy are destroyed, since in some forms, *e.g.*, Neritina, the development does not proceed far enough to introduce such a struggle. From the very beginning of development the ova are divided into two classes, those which segment regularly and develop into normal embryos, and those which divide irregularly and never form embryos at all. Blochmann thinks that in Neritina the eggs which do not develop have not been fertilized, while McMurrich believes that too little yolk was furnished for the number of eggs produced, and that, therefore, some of the eggs broke down and were used as food by the embryos which survived. "This process," he says, "might have been seized upon by natural selection, and increased by it until it became a regular process of development."

I am inclined to believe that in different species different causes may have been operative in producing these abnormal forms. In Neritina, Purpurea and all other forms in which the development of some of the ova goes no farther than a few irregular cleavages, the most probable cause of such non-development seems to be the lack of fertilization, for if McMurrich's supposition is the correct one we should expect to find the ova which undergo development larger than those which do not, but there is no evidence of such disparity in size. On the other hand, in those forms in which the abnormalities do not appear at an early stage and with great regularity, *e.g.*, Crepidula or Urosalpinx, in which they may or may not be present, and if present may occur at any stage, in such cases I am convinced that the abnormal forms are the result of unfavorable environment, *e.g.*, lack of oxygen, presence of bacteria, mechanical pressure, etc.

C. HISTORY OF THE CLEAVAGE.

NOMENCLATURE.

The question of an accurate and convenient nomenclature for the various cells of the cleaving ovum, while of no scientific value, is, nevertheless, of considerable practical importance.

Almost every writer on cleavage has a nomenclature of his own, and not only must one learn a new system every time he reads a new paper, but the difficulties of comparing the work of one author with that of another become constantly greater and greater. If it were possible to invent a system, as some have attempted to do, which would be simple, convenient, and *universally applicable*, it could, and of course would, be accepted by every one who writes upon this subject; but the differences in cleavage are so great that such a consummation seems to me almost hopeless. Besides, there are peculiar features in the cleavage of every egg upon which nature seems to lay emphasis, and such features deserve some special recognition in the nomenclature. Perhaps the most serious objection to any of the systems of nomenclature which have been proposed is the fact that it is almost impossible to recall cells by letters and figures when they differ from each other only in the value of one out of many exponents, *e.g.*, it is practically useless for an ordinary reader to attempt to remember the differences in the position, shape, and history of the cells called b''_2 and b'''_2 of Blochmann's ('81) system, or d^1 and $d^{1.4}$ of Wilson's ('92), whereas it is comparatively easy to recall these cells if they are known as the basal and terminal cells in the posterior arm of the cross. It is not always possible to designate cells by colloquial names which shall be of any help in forming a mental image of them, but wherever it is possible it should be done. At the same time some brief and accurate system of nomenclature is necessary in order to show the derivation of cells, and also for the purposes of comparison and reference.

I have, therefore, concluded to employ, so far as possible, a double system of names for every blastomere, one of which shall be, if you please, its common name, the other its scientific designation. Regarding the latter, which alone needs to be mentioned in this place, I shall, in the main, follow Wilson's system, given in his work on "The Cell Lineage of Nereis," modifying it only to this extent, that the quartettes [1] of cells, separated at various times from the macromeres will be desig-

[1] I use the term *quartette*, as employed by Kofoid ('94), to designate a group of four cells of the same generation, one of which belongs to each of the quadrants

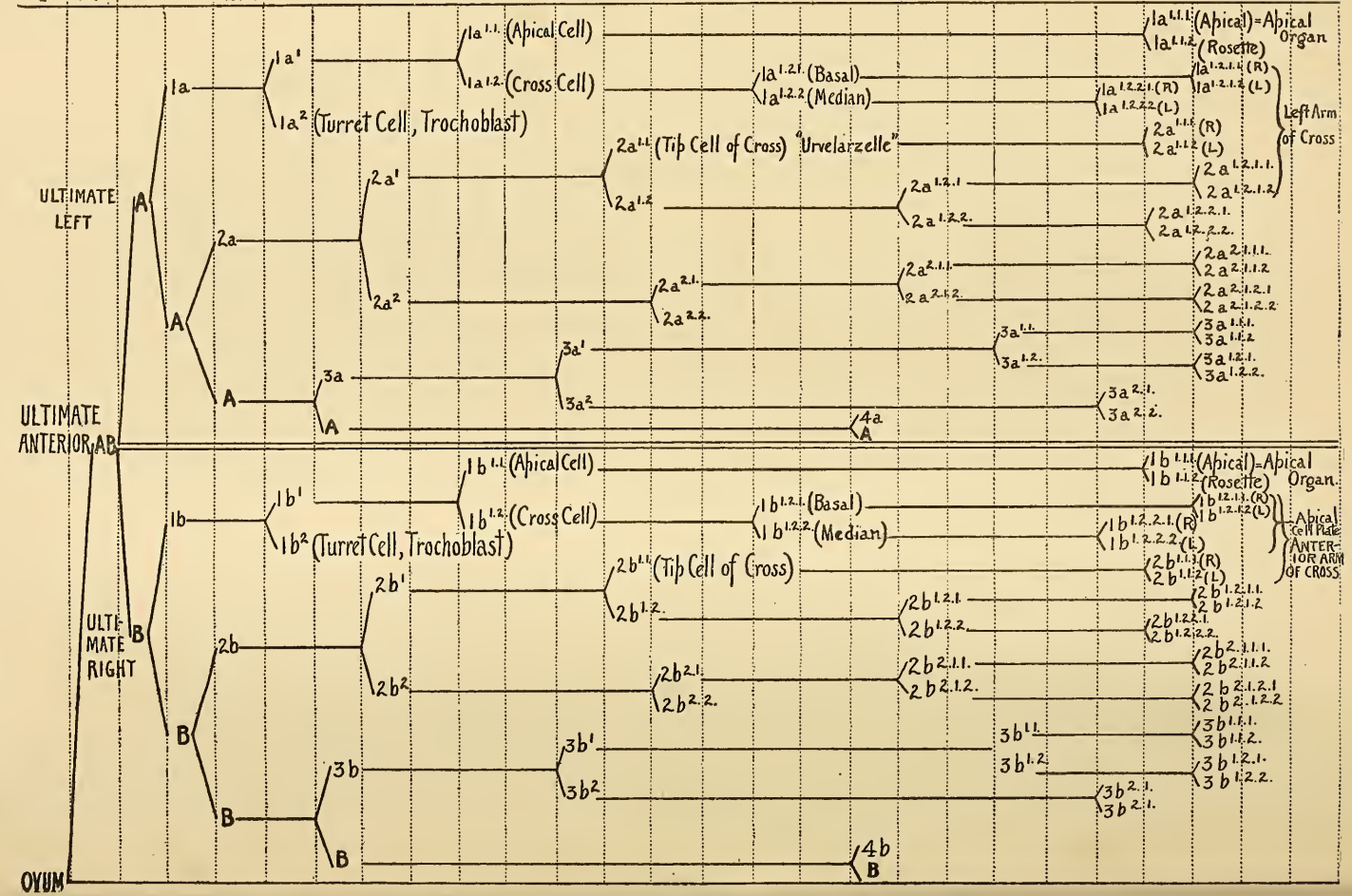
nated by coefficients rather than by exponents ; *e.g.*, the first quartette of micromeres and all their derivatives are designated by the coefficient 1 (1a, 1d, $1a^{1.2}$, $1c^{2.2}$, etc.), the second quartette and its progeny by the coefficient 2 (2a, 2d, $2c^{3.1}$, etc.), the third quartette by the coefficient 3 (3a, 3d, etc.), and the fourth quartette by 4 (4a, 4d, etc.). I emphasize this difference between the quartettes of micromeres because in general their histories are very different, and also because it is only by following the different quartettes that I have been able to trace the cell lineage in the more advanced stages.

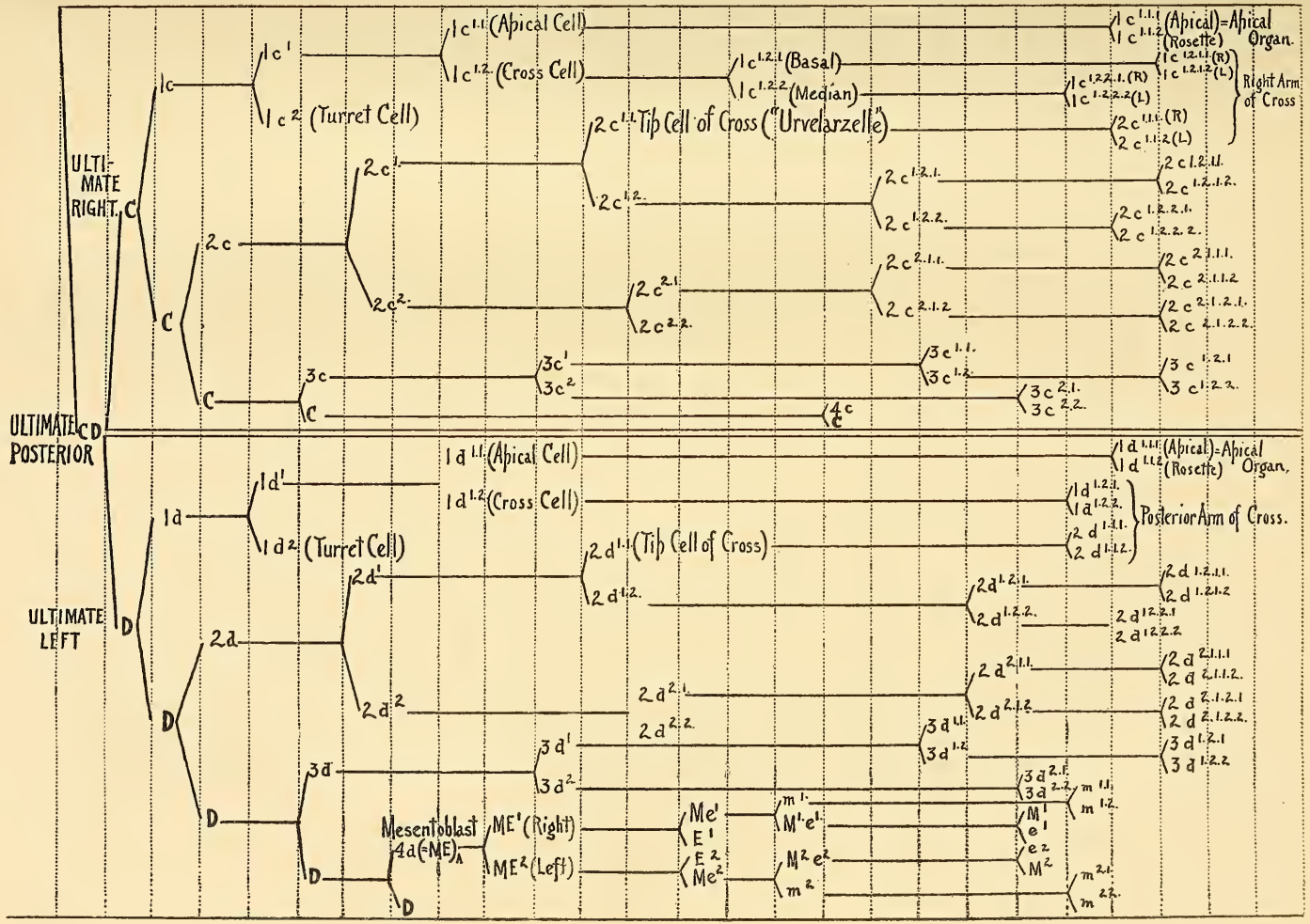
Another and an all-sufficient reason for emphasizing in the nomenclature the different groups or quartettes separated from the macromeres, is the fact that, so far as known, the same number of quartettes with essentially the same destiny is separated in all annelids and mollusks with holoblastic segmentation. This is certainly a feature of great morphological importance, and deserves special recognition in the nomenclature. This system of nomenclature will be better understood by reference to the following cytogenetic table.

The animal and vegetal poles are considered the fixed points in the egg. In the ectoblast the stem or parent cell is in all cases the upper one. The stem cell in the entoblast and mesoblast is in every case the lower one. If, in any case, the cleavage is perfectly meridional (an exceedingly rare thing), the right moiety is considered the stem cell. The terms *right* and *left* are employed in the usual sense, *i.e.*, right is clockwise, left is anti-clockwise. A cleavage is oblique to the right, or, following Lillie ('95), *dexiotropic*, when the upper moiety lies to the right of the lower ; it is oblique to the left, or *laeotropic*, when the upper moiety lies to the left of the lower. The direction of a cleavage refers to the direction of the nuclear spindle, not to the plane of the division wall.

of the egg. In numbering the different quartettes, however, I have departed somewhat from Kofoid's system. The four macromeres are the basal quartette ; the first group of ectomeres separated from these are the first quartette, the second group the second quartette, etc.

TABLE OF THE CELL-LINEAGE OF CREPIDULA.




ULTIMATE RIGHT, C
ULTIMATE POSTERIOR CD
ULTIMATE LEFT
1c
1c¹
1c²
1c¹·¹ (Apical Cell)
1c¹·² (Cross Cell)
1c¹·²·¹ (Basal)
1c¹·²·² (Median)
1c¹·¹·¹ 1c¹·¹·² (Apical)=Apical (Rosette) Organ.
1c¹·²·¹·¹ (R) 1c¹·²·¹·² (L)
1c¹·²·²·¹ (R) 1c¹·²·²·² (L)
Right Arm of Cross
2c
2c¹
2c²
2c¹·¹ Tip Cell of Cross ("Urvelarzelle")
2c¹·² 2c²·¹ 2c²·²
2c¹·²·¹ 2c¹·²·² 2c²·¹·¹ 2c²·¹·² 2c²·²·¹ 2c²·²·²
2c¹·¹·¹ 2c¹·¹·² 2c¹·²·¹·¹ 2c¹·²·¹·² 2c¹·²·²·¹ 2c¹·²·²·² 2c²·¹·¹·¹ 2c²·¹·¹·² 2c²·¹·²·¹ 2c²·¹·²·² 2c²·²·¹·¹ 2c²·²·¹·² 2c²·²·²·¹ 2c²·²·²·²
3c
3c¹ 3c²
3c¹·¹ 3c¹·² 3c²·¹ 3c²·²
3c¹·¹·¹ 3c¹·¹·² 3c¹·²·¹ 3c¹·²·² 3c²·¹·¹ 3c²·¹·² 3c²·²·¹ 3c²·²·²
4c
1d
1d¹
1d²
1d¹·¹ (Apical Cell)
1d¹·² (Cross Cell)
1d¹·¹·¹ 1d¹·¹·² (Apical)=Apical (Rosette) Organ.
1d¹·²·¹ 1d¹·²·² 2d¹·¹·¹ 2d¹·¹·² Posterior Arm of Cross.
1d² (Turret Cell)
2d¹·¹ (Tip Cell of Cross)
2d
2d¹
2d²
2d¹·² 2d²·¹ 2d²·²
2d¹·²·¹ 2d¹·²·² 2d²·¹·¹ 2d²·¹·² 2d²·²·¹ 2d²·²·²
2d¹·¹·¹ 2d¹·¹·² 2d¹·²·¹·¹ 2d¹·²·¹·² 2d¹·²·²·¹ 2d¹·²·²·² 2d²·¹·¹·¹ 2d²·¹·¹·² 2d²·¹·²·¹ 2d²·¹·²·² 2d²·²·¹·¹ 2d²·²·¹·² 2d²·²·²·¹ 2d²·²·²·²
3d
3d¹ 3d²
3d¹·¹ 3d¹·² 3d²·¹ 3d²·²
3d¹·¹·¹ 3d¹·¹·² 3d¹·²·¹ 3d¹·²·² 3d²·¹·¹ 3d²·¹·² 3d²·²·¹ 3d²·²·²
Mesentoblast 4d(=ME)A
ME¹ (Right)
ME² (Left)
Me¹ E¹ E² Me²
m¹·¹ M¹e¹ M²e² m²
M¹ e¹ e² M²
m¹·¹ m¹·² m²·¹ m²·²

The Unsegmented Ovum (Fig. 1).

The spermatozoa meet the ova in the oviduct and are inclosed with them in the egg capsules, but the maturation and fecundation of the ova do not take place until after the capsules have been laid. I have reserved for another paper the study of the nuclear phenomena which underlie these processes.

Two polar bodies are extruded : they are clear and vesicular, and each contains a small nucleolus-like sphere of chromatin. The chromatin in the first-formed polar body usually divides, though the body itself frequently does not. Both polar bodies remain attached exactly at the centre of the ectodermal area, frequently until the ectoderm cells have extended more than halfway around the egg, Figs. 49 and 50. In all these later stages the chromatin is not surrounded, as in earlier stages, by a clear vesicular layer of cytoplasm, but seems to have dissolved and spread throughout the whole body, so that it stains quite uniformly. Sooner or later the polar bodies fall off and disappear, and in sections of embryos of the stage shown in Fig. 93, I have, in several cases, found them in the mesenteron, having been drawn in with the nutrient fluid surrounding the embryos.

The unsegmented ovum is nearly spherical in C. plana and C. fornicata, though in the larger eggs of C. convexa and C. adunca it is generally elongated in one diameter, so that when seen from either pole the outline is elliptical.[1] The protoplasmic portion in all of these species is small as compared with the yolk, but much smaller relatively in the last two than in either of the first two. There is no sharp boundary line between the protoplasmic and deutoplasmic portions ; on the contrary, the former sends out pseudopodia-like branches between the yolk spheres, and the spheres themselves grow smaller and more indistinct as one approaches the protoplasmic portion. Fig. 1, the earliest stage drawn, shows the male and female pronuclei lying close together but still distinct. Each nucleus contains, besides several bands or loops of chromatin, a homogeneously staining nucleolus of considerable size, from which the bands of chromatin seem to radiate. In the figure the female pro-

[1] McMurrich has pointed out that the eggs of Fulgur are elongated.

nucleus is slightly larger than the male, and this continues to be true as long as the pronuclei can be recognized as such.

Two polar bodies are shown in the figure at the upper pole, of which the first formed is much the larger ; the chromatin in this has already divided into two masses, though the cell body is still undivided.

At the vegetal pole of the egg there is frequently found a rounded mass of hyaline substance, which stains homogeneously. It persists until after the first two cleavages, lying in the furrow between the macromeres, but apparently attached to one of them only. I am not satisfied as to the significance of this body, but am inclined to believe that it is a remnant of the stalk of attachment by which the ovum was fastened to the basal membrane of the ovarian follicle. If this view be correct, the polarity of the egg is determined in the ovary, the vegetal pole lying next the membrane, the animal pole next the lumen of the follicle. This is precisely the condition in Unio (Lillie ('95), p. 10), where the point of attachment marks the position of the micropyle. There is no micropyle in Crepidula, and no need of any, since there is no egg membrane, but this hyaline mass suggests the micropyle, not only because it is located at the vegetal pole, and seems to be formed in the same way, but still more remarkably, because the spermatozoan usually, though not invariably, enters the egg at this spot. In all cases the polarity of the egg is definitely established long before the polar bodies are formed, and if my interpretation of the hyaline mass is correct, the animal and vegetal poles of the egg are established at a very early stage in the ovary.[1]

[1] Since the above was written a brief study of the eggs of Fulgur carica and of Sycotypus canaliculatus shows that a similar body, though very much larger than that in Crepidula, is present in these animals. In both Fulgur and Sycotypus this body contains a considerable amount of yolk and yet stains quite uniformly, as it does in Crepidula.

I am convinced that this peculiar body is homologous with the problematical lobe which is described by Mead ('95) in the egg of Chaetopterus, and further, it is probably identical with the polar rings observed by Whitman ('78) in Clepsine, and since then by various authors in different annelids.

I. THE PRIMARY CLEAVAGES.

1. *The First Cleavage. Figs. 2–6.*

I cannot state exactly the length of time which intervenes between fertilization and the first cleavage, nor between the latter and the following cleavages. However, not less than four hours elapse after the entrance of the sperm into the ovum before the first cleavage begins, and the interval is probably longer. I have frequently found Crepidulas in the process of egg-laying, and after carrying the newly laid eggs several miles to the laboratory and there fixing and staining them, have found on examination that the male and female pronuclei were still far apart.

No "segmentation nucleus" is formed, *i.e.*, the male and female pronuclei do not fuse before the appearance of the karyokinetic spindle which introduces the first cleavage. In fact, the male and female chromatin loops remain separate until the equatorial plate stage of the first spindle. About the stage shown in Fig. 2, however, the chromatin loops form a continuous plate ; though the part of the plate lying beneath the polar bodies (the upper side in the figure) probably came from the female pronucleus, while the other portion (the lower half) came from the male pronucleus. The axis of the spindle lies in the long diameter of the protoplasmic area, or rather the protoplasmic area continually enlarges its diameter in the direction of the axis of the spindle from the time the spindle first appears until the first cleavage is completed. The radiations of the archoplasmic bodies at the poles of the spindle are plainly visible in all the surface views, and a large central corpuscle or centrosome can be seen in the most favorable preparations. After the chromatin is distributed equally to the two poles of the spindle the division of the cell body begins. A furrow first appears at the formative pole, and gradually extends until it forms a constriction all around the ovum, but deeper at the formative pole than elsewhere, Figs. 3 and 4. The cell body then divides into two equal portions *AB* and *CD*, Figs. 5 and 6. These blastomeres are at first nearly spherical,

and touch each other only by a comparatively small surface; later they become much more closely pressed together, and the surface by which they are in contact becomes much larger, so that each of the blastomeres is almost a hemisphere, Fig. 7.

Immediately after the division of the nucleus the archoplasmic bodies, Figs. 5 and 6, begin to increase in size and to become much more definite in outline. Each one lies close beside the nucleus in the position of the pole of the preceding spindle, and in surface preparations looks as if it might be the shadow of the nucleus. My attention was first called to these bodies by finding what I supposed to be two nuclei in each cell, one of which was fainter in color and outline than the other and looked as if it might be at a lower level in the egg, and it was some time before I could bring myself to believe that these bodies, which are so plainly visible even in preparations of the whole egg, and which in many cases are fully as large as the nuclei themselves, were nothing other than the "archoplasmic bodies" of Boveri or the "spheres attractive" of van Beneden.[1]

A careful study of one of these bodies in its resting stage shows that it is a clear vesicular structure, containing apparently a finely granular fluid and having a fairly definite outline from which radiations proceed in every direction, Figs. 5, 6, 8, 10, etc. As it begins to divide, however, preparatory to the formation of the karyokinetic spindle, the definite outline of the body grows fainter and fainter until it cannot be recognized, while the radiations extend much further through the protoplasm of the cell.

At the close of the first cleavage, the nuclei, asters, and protoplasmic areas lie directly opposite each other in the two blastomeres, Fig. 5, but as soon as the blastomeres begin to flatten against each other and the whole egg assumes a more compact form all these structures move in the direction of a clock's hands, as shown in Fig. 6. *This movement of the nuclei, asters, and protoplasm takes place invariably in the same direc-*

[1] I shall throughout this paper call these bodies the *asters*, a name first used in this connection by Fol ('73) to signify the radiating cytoplasmic structure within the cell.

tion, and it must therefore have been predetermined during, and perhaps before, the first cleavage.

I have not been able thus far to discover by what means or in what manner this movement is predetermined. In Crepidula the first spindle does not seem to indicate any such rotation, though it is exceedingly suggestive to note that Warneck ('50) in the case of Limax and Fol ('75) in Cymbulia found that the first cleavage was oblique to the axis of elongation of the egg. Kofoid ('95) however, in his recent careful work on Limax, found no evidence in favor of Warneck's account. In some cases in which the first cleavage is very unequal, as *e.g.*, in Urosalpinx, the plane of the first cleavage is oblique to the axis of elongation, and it may be that it is also oblique to the polar axis of the egg.

But however the direction of these movements may be predetermined, the fact that they are predetermined, at least during the period of the first cleavage, is a profoundly significant one, indicating as it does that *the first cleavage of the egg belongs to a series of "spiral" cleavages which for at least nine successive generations of cells are alternately dexiotropic and laeotropic.*

Strictly speaking the first cleavage could scarcely be called a *spiral* one, since there is but a single spindle which intersects the chief axis of the egg; and besides there is no definite cross axis to which the direction of this spindle can be referred. It is certain, however, that the dexiotropic turning of the nuclei and protoplasmic areas after the first cleavage is, on the one hand, causally related to their laeotropic turning during the second cleavage, and on the other hand it seems to be predetermined at least as early as the preceding cleavage. It is therefore highly probable that the first cleavage belongs in the same category with the succeeding spiral cleavages, though perhaps it would be more exact and less paradoxical to speak of it as *prospectively spiral and dexiotropic.*

These so-called "spiral" cleavages are always radially symmetrical.[1] A glance at Fig. 6 or 7 will show that the two blastomeres are not mirrored representatives of each other, *i.e.*,

[1] This subject is treated at length in the concluding section of this paper.

the egg is not bilaterally symmetrical with reference to the first cleavage plane, but it is radially symmetrical ; the blastomeres are congruent antimeres, and the egg at this stage is a "one-rayed radiate," as Chun ('80) calls the Ctenophores. The radial symmetry of the egg prevails undisturbed from the time polarity is first established (p.39) until the primary mesoblast is formed (p.67). After this event the posterior half of the egg becomes more or less bilateral, while the anterior half remains radially symmetrical. Finally, at a relatively late stage the entire egg becomes bilateral.

The rotation of blastomeres in some of the later stages of cleavage has long been known and commented upon. So far as I can find Selenka ('81) first used the term *spiral* in this connection. He described in the polyclades a "laeotropen oder λ-Spirale" in the formation of the first quartette of micromeres, and a "dexiotropen oder δ-Spirale" in the formation of the second quartette, but he did not apply either of these terms to the earlier or later cleavages. Lang ('84) first called attention to the fact that the *second cleavage* in Discocoelis takes place in a "left-wound spiral." Since then this same fact has been observed in the case of many other animals (cf. Conklin ('91), Wilson ('92), Heymons ('93), Lillie ('95), *et al.*), and, with one or two exceptions which will be described later, the direction of this cleavage is invariably the same.

Up to the present, however, no one has shown that the *first cleavage* also is a spiral one. In all other works on this subject, so far as I am aware, it is asserted that the position of the spindles during the second cleavage is the first indication of spiral cleavages (see Wilson ('92), pp. 387, 453, Heymons ('93), p. 249, Lillie ('95), pp. 14, 15).

I believe, however, it may be safely asserted that in all cases in which the second cleavage is laeotropic the first is dexiotropic, and that the initial cause of the spiral cleavages is not to be found in the direction of the nuclear spindles, but rather in the structure of the unsegmented egg itself.

2. *The Second Cleavage. Figs. 7–10.*

The spindles usually appear simultaneously in the two blastomeres, Figs. 7, 9, though occasionally earlier in one than the other, as shown in Fig. 8. The axes of the two spindles are almost parallel to each other, and at right angles to that of the preceding spindle. The two spindles are not quite parallel, however, as is shown in Fig. 7, where the spindles are laeotropic, the left pole in each case being at a higher level in the egg than the right one. Thus the axes of the spindles, when viewed from the side, cross each other at a slight angle. It will also be noticed in Fig. 7 that the entire spindle in each blastomere lies somewhat to the left of the median plane of the blastomere. The position and direction of the spindles in this case indicate, before the division occurs, that the cleavage will be laeotropic. The spiral character of the preceding cleavage could be observed only after the division had occurred.

The first cleavage furrow is at first a straight line as seen from the animal pole, Fig. 6, but as the second cleavage comes on, this line becomes bent slightly to the right when placed in the line of vision, Figs. 7, 9. From the angles where this bent portion joins the rest of the first furrow, the two halves of the second cleavage run outward toward the periphery, Figs. 9, 10. The second cleavage really consists of two quite independent furrows ; their ends never meet at the centre, and one of them may appear somewhat earlier than the other, Fig. 8. These furrows begin to form near the animal pole and run out around and through the blastomeres until they reach the vegetal pole, completely dividing the two blastomeres into four, which are approximately equal in size.

3. *The Origin and Significance of the Polar Furrows. Figs. 7–12, Diagram 2.*

The bent portion of the first furrow included between the central ends of the second cleavage is a feature of considerable practical as well as theoretical importance. It is a well-known fact that there is, in the eggs of many animals, a furrow at the intersection of the first and second cleavage planes, which does

not lie in either of these planes, but is oblique to both of them. Rabl ('79) calls this in Planorbis the "cross" or transverse furrow ("Querfurche"), indicating thereby that it lies transverse to the long axis of the embryo. Blochmann ('81) also mentions this furrow as being present in the egg of Neritina, and describes the method of its origin. He calls attention to the fact that it lies in the transverse plane of the embryo; and he considers that it is caused by the difference in the time of division of the two cells. But that this is not generally the case, is shown by the fact that it is present in many eggs in which the division of the first two blastomeres occurs simultaneously. Rauber ('82) has described at some length a similar furrow, which is found in the frog's ovum, as well as in Petromyzon and Gobius. He calls it the breaking line ("Brechungslinie"), and says that it may be formed in two ways: (1) the second furrow really consists of two furrows, one of which divides one of the first two blastomeres, the other the other one; these two furrows may or may not meet in the centre; in the latter case the breaking line is formed; (2) if a breaking line is not formed at first, it may appear later by the shifting of the blastomeres. While Rauber considers that the position of the breaking line has an influence on the subsequent cleavage, he regards its position relative to the other furrows or to the embryonic axes as purely a matter of chance. As he points out, it is particularly well marked in the four-cell stage of many ova; at this stage there are often two "cross furrows" on opposite sides of the egg; these are at right angles to each other, so that each of the four cells is acute at one pole and truncated at the other. O. Hertwig ('80) has also called attention to this furrow in the egg of Sagitta. He says of it: "An dem animalen Pole des Eies, welcher gerade abgebildet ist, stossen nicht alle vier Zellen, wie es bei regelmässiger Furchung der Fall sein sollte, in einem Punkte zusammen, sondern nur zwei derselben berühren sich mit verbreiterten Enden und bedingen eine kurze gerade Furche, welcher wir ihrer Lage nach als *Polarfurche* benennen wollen; die beiden anderen Zellen, welche von der gegenseitigen Berührung ausgeschlossen sind, enden zugespitzt an den beiden

Enden der Polarfurche. Ganz dieselben Verhältnisse wiederholen sich am vegetativen Pole ; nur treffen sich hier die beiden Zellen, welche den animalen Pol nicht erreichten, mit verbreiterten Enden. Sie bilden eine *vegetative Polarfurche*, welche die animale, wenn wir beide auf dieselbe Ebene projiciren, unter rechtem Winkel kreuzt, wie man beim Wechseln der Einstellung an dem durchsichtigen Object leicht feststellen kann. . . . Eine ähnliche Anordnung der vier ersten Furchungszellen wie bei Sagitta hat soeben auch Rabl an den Eiern von Planorbis genau beschrieben, er nennt die Polarfurche Querfurche und bemerkt hierzu, dass sie einen wichtigen Anhaltspunkt für die Orientirung des Keimes abgiebt."

In all holoblastic eggs which are laden with yolk the polar furrow at the vegetal pole is much longer than the one at the animal pole, — in fact, the latter may be absent altogether, as is the case with Crepidula. In using the expression "polar furrow" in connection with this animal, it must be understood to refer only to that structure which Hertwig calls "vegetative Polarfurche." As just remarked, the name "Querfurche" seems to have been given in the belief that this furrow is always transverse to the antero-posterior axis of the embryo, as it is in Planorbis and Neritina, and as I have found is the case in Urosalpinx and Tritia. If one may judge from the figures alone this seems to be its position in Nassa and Fusus, as described by Bobretzky ('77), and in Vermetus, studied by Salensky ('87). In all forms, however, in which the first cleavage coincides with the antero-posterior axis, or is at right angles to it, the furrow in question could not be transverse to that axis, but would necessarily be oblique to it; this is its position in Nereis, Umbrella, and Crepidula. In such cases the name "cross furrow" is evidently a misnomer. The furrow bears no constant relation to the axes of the embryo, being at one time transverse and at another oblique to the longitudinal axis ; and it is just as illogical to name this furrow from its relation to the axes of the embryo as it would be to name the first cleavage from such a relation, which in some animals coincides with the antero-posterior axis, in others is at right angles to it, and in still others is oblique to it.

There are also objections to the word "Brechungslinie," proposed by Rauber; it is not a breaking line, nor a broken portion of a line, and the name indicates nothing with regard to its position. Moreover the fact that the "Brechungslinie" is not constant in position indicates that it is not the result of a determinate series of spiral cleavages, as is the case among annelids and mollusks, but that it is merely a "pressure surface," the result of surface tension, and it therefore has no reference to the character of the cleavage, which might be radial or bilateral or neither. This term, therefore, even if unobjectionable for the purpose for which it was employed by Rauber, ought not to be applied to the furrow in question.

The expression "polar furrow," however, is open to none of the objections mentioned; this furrow is found only at the two poles of the egg, and so far as the name is descriptive at all, it is quite accurate. I shall, therefore, use it exclusively hereafter to designate that portion of the first furrow which lies between the central ends of the second furrow, both at the animal and vegetal poles. Although always and entirely a part of the first furrow, it seems to lie in, and form a part of, both the first and second furrows.

Although in different animals the polar furrow may bear no constant relation to the embryonal axes, it does in all known cases of spiral cleavage bear a very constant relation to the first and second cleavages. In Crepidula, for example, if the first furrow be placed in the line of vision, the polar furrow always bends to the right, in the second furrow it bends to the left, and this is true whichever end of the furrow is nearer the observer. These relations are true only when the egg is viewed from the animal pole; obviously they would be reversed if seen from the vegetal pole, *i.e.*, the polar furrow would bend to the left when in the first furrow and to the right when in the second. This relation is of great practical importance, since it enables one to distinguish at a glance the first furrow from the second, even up to an advanced stage, and it thus forms a ready means of orientation. In Fig. 10 and all succeeding stages it is impossible to distinguish between the first and second furrows except in this way; in Figs. 8 and 9, how-

ever, the second cleavage is not yet complete, and can, therefore, be easily distinguished from the first, and in the ova which are there figured, as well as in hundreds of others which I have studied, the relation of the polar furrows to the first and second cleavages is always the same.

Similar furrows are shown and described in the works of very many authors, and indeed in the ova of almost every group of animals ; but in most cases no mention is made of any definite relation between these furrows and the first and second cleavage planes. In the frog, according to Rauber ('82), this furrow bears no constant relation to the first two cleavages, and Eycleshymer ('95) seems to have found the same thing true of Amblystoma, Petromyzon, and Corregonus. But in a very large number of animals, belonging to groups as far removed from each other as mollusks, annelids, and polyclades, the relation between the polar furrow and the first and second cleavages is a constant one. In Blochmann's figures of the egg of Neritina, and in Lang's figures of Discocoelis, the polar furrow is shown bending to the right in the first cleavage (the position which it has in Crepidula), though neither of these investigators calls attention to this fact in the text or description of figures.[1] The same fact is further shown and commented on by Wilson ('92) in the case of Nereis, Heymons ('93) in Umbrella, and Lillie ('95) in Unio. A very striking exception to this rule has been discovered by Crampton ('94) in the case of Physa, a sinistral gasteropod, in which the direction of the polar furrow is reversed, and he points out the fact that the figures which Rabl ('79) gives for Planorbis, and a figure given by Haddon ('82) for Janthina, seem to show a similar reversal. So far as I know these are the only cases on record in which the polar furrow constantly turns to the left when seen in the first furrow, whereas in *many* cases, as I have indicated, it constantly turns to the right.[2]

[1] One figure which Blochmann gives, Fig. 40, corresponds very closely with my Figs. 9 and 10; the second furrow is still incomplete, and two of the macromeres are much more obtuse at the centre than the other two. The polar furrow thus formed bends to the right in the first furrow just as it does in Crepidula.

[2] Since this was written Kofoid's final paper on Limax ('95) has appeared, in which he thoroughly discusses the "cross furrows," especially the relation of the

A phenomenon so widespread and so striking cannot be wholly adventitious and without significance. As we have seen, Blochmann explains the formation of the polar furrow in Neritina by the fact that one of the first two blastomeres divides before the other one. This would not explain the constant relation of the polar furrow to the first and second cleavages unless in all the groups mentioned one blastomere divided earlier than the other one, and this of course is not the case.

Rauber ('82) attributes the formation of the "Brechungslinie" to a tendency on the part of all the furrows to avoid the pole. This, of course, is not true of the first furrow, and in any case it is no explanation of the phenomenon. Jordan and Eycleshymer ('94) are right when they say (p. 412), "The furrows do not *avoid* the pole ; but the mechanical cell-stresses are rarely so adjusted that the furrows intersect at the pole. There seems no need for a special term — 'Polflucht' — to express this fact, since the 'shunning' of the pole can hardly be a matter of primary significance." But while surface tension is a sufficient causal explanation of such pressure surfaces as the "Brechungslinie," this principle alone is not able to explain the *constant* position of the polar furrow with reference to the first two cleavages, and this constant position is a matter of primary significance.

In his classical work on Nereis, Wilson ('92) has carefully described the polar furrows, and has pointed out the fact that they are of great value in the orientation of the egg and embryo.[1] The position of these furrows is precisely the same in Nereis and Crepidula, except that there is a short polar furrow at the upper pole in Nereis which is generally wanting in Crepidula. In the last section of his paper Wilson points out the significance of the "cross furrow," and although he does not directly explain the cause of its constant relation to

ones on the dorsal and ventral sides of the egg. As my account is in substantial agreement with Kofoid's, and as it touches upon a few points not mentioned by him, I have allowed it to stand as first written.

[1] I had earlier ('91) called attention to the fact that the polar furrow bears a constant relation to the first two cleavages, but had attempted no explanation of this fact.

the first two cleavages, yet that explanation lies so near the surface that I should not take the trouble to enter upon that subject here were it not for the fact that I have a few suggestions to make which are not found in his work.

The polar furrows are in all cases the result of spiral cleavages, and the direction of the polar furrows relative to the first and second cleavages is always dependent upon the direction of the spirals. Because the second cleavage is laeotropic, the vegetative polar furrow bends to the right in the first cleavage and to the left in the second ; in Physa, in which the direction of the spirals is reversed, the direction of the polar furrows is reversed.

The cause of these relations can be made plain by means of the accompanying diagram. In Diagram 2, *a*, the macromeres B and D lie at a slightly lower level than A and C, and have given off A and C by a laeotropic division. It is seen in this figure that there is but one polar furrow, and that it turns to the right when seen in the first furrow, and to the left when seen in the second. This is the state of affairs which prevails in Crepidula, Neritina, Umbrella, Urosalpinx, etc. Let us suppose, however, that the passage from the two to the four-cell stage had taken place in the reverse direction as it does in Physa, and as is shown in Diagram 2, *b*, where A and C lie at a somewhat lower level than B and D, and have given off the latter by a dexiotropic division. There is here but one polar furrow, and when seen in the first furrow it turns to the *left ;* when in the second furrow, to the *right.* It is evident, therefore, in all those cases where there is but one polar furrow which turns to the right when seen in the first furrow, and to the left when seen in the second, that the second cleavage was laeotropic.

As a rule when there is but one polar furrow, it is somewhat shorter at the formative than at the vegetative pole, Diagram 2, *c*. Yet as an extreme case there are found ova in which the single polar furrow is almost equal in length at the two poles ; this is admirably illustrated by the egg of C. convexa, Diagram 2, *a*, which is laden with a large quantity of yolk, and in which the macromeres A and C, while lying at a slightly higher level than

B and D, are somewhat smaller in size, while the single polar furrow remains almost as long at the animal as at the vegetal pole. In the egg of C. fornicata, which contains less yolk,

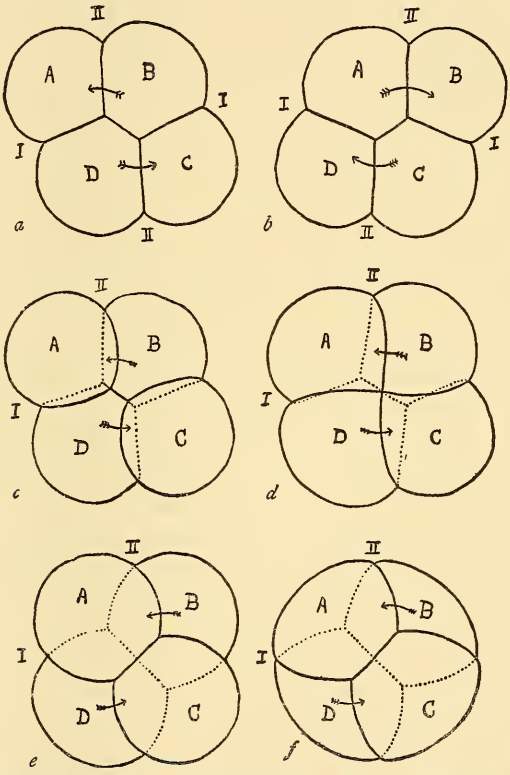


DIAGRAM 2. — Polar furrows of spiral cleavage. — *a*, The condition in eggs with much yolk (C. adunca), in which the polar furrow is as long at one pole as at the other. — *b*, The position of the polar furrow in reversed cleavage. — *c*, The condition in eggs with a smaller amount of yolk (C. fornicata), in which the cells A and C lie at a higher level than B and D, and the polar furrow is shorter at the animal than at the vegetal pole. — *d*, Egg with a still smaller amount of yolk (C. plana), in which the four macromeres meet in a point at the animal pole; there is but one polar furrow, the vegetal. — *e*, Egg of Discocoelis (Lang), in which there are two polar furrows of nearly the same length. — *f*, Egg of Botryllus; polar furrows similar to the last, but whole egg more compact.

and which is represented in Diagram 2, *c*, the relation of the blastomeres to each other is in the main the same as in Diagram 2, *a*, still the macromeres A and C overlie B and D to a greater extent than in the preceding diagram, and there-

fore the polar furrow, while running in the same direction at both poles, is distinctly shorter at the animal than at the vegetal pole. Diagram 2, *d*, represents the condition of the polar furrow in C. plana ; it shows that in this egg, which has less yolk than that of C. fornicata, the blastomeres A and C overlie B and D still more than in the case last mentioned, and that they meet in a *point* at the animal pole. There is here no polar furrow at all at the animal pole, though the one at the vegetal pole is well developed. In Diagram 2, *e*, which is a diagrammatic representation of the egg of Discocoelis as described by Lang, the macromeres A and C not only overlie B and D, but they meet in a line, which forms a polar furrow at the animal pole lying at right angles to the one at the vegetal pole. These furrows may or may not be equal in length ; generally the one at the animal pole is the shorter, though Lillie has found that it is the longer in Unio, which is due to the fact that in this case the cells at the animal pole are larger than those at the vegetal. Finally, in Diagram 2, *f*, which represents the egg of Botryllus, we find the greatest degree of compactness of the blastomeres ; the polar furrows at the upper and lower poles are nearly equal in length, and the individual blastomeres no longer preserve independence of outline, but are rounded into a nearly perfect sphere. Two or more of these different forms may be found at different stages in the cleavage of the same egg. At the moment of cleavage the blastomeres are generally more independent and less compact than during the "resting stages" between cleavages. Thus in many ova the blastomeres at the moment of cleavage are like those represented in Diagram 2, *e*, while during the "resting period" they become much more compact, like those shown in Diagram 2, *f*.

Two types of ova are represented in the diagram given above, one in which there is scarcely any polar differentiation, the other in which it is well pronounced. The former is represented by figures *e* and *f*, and in such cleavage forms polar concentration of protoplasm and nuclei is impossible, the nuclei in fact lie near the centres of the blastomeres and the yolk is uniformly distributed throughout the protoplasm ; the latter type is represented

by figures *a* to *d* in which the polar concentration of protoplasm and nuclei is very marked. In all eggs in which there is but one polar furrow there is decided polar differentiation of the yolk and protoplasm ; where two polar furrows are present this segregation is less pronounced.

The statement that the polar furrow turns to the right when seen in the plane of the first cleavage is true only when there is one polar furrow, and that the one at the lower pole. When there are two polar furrows, as in Diagram 2, *e* and *f*, the lower one still preserves this same relation when seen from the animal pole, while the upper one bends to the *left* when seen in the first furrow, and to the *right* when seen in the second. Of course, if these were viewed from the vegetal pole, the relations would be reversed.

The fact that in very many cases the first cleavage is dexiotropic and the second cleavage laeotropic is a profoundly important and significant one, determining as it does, not only the direction and relation of the polar furrows, but *also influencing more or less the character and direction of every succeeding cleavage. They are the first of a long series of spiral cleavages which take place alternately to the right and to the left, each of which, except the first, finds the sufficient cause of its direction in the direction of the preceding cleavage.*

4. *The Axial Relations of the First Two Cleavages.*

Throughout the course of segmentation the four macromeres remain very much larger than the cells to which they give rise, and as they do not change their relative position, at least until about the time of the closure of the blastopore, it becomes very easy to orient all the future furrows and cells with reference to the first two cleavages. If we examine one of the later stages, such as Figs. 61 and 64, in which the antero-posterior axis of the embryo is well marked by the elongated blastopore, we find that the four macromeres and the polar furrow are still recognizable, and that the cleavage line in which the polar furrow bends to the right, *i.e.*, the first cleavage, is transverse to the antero-posterior axis of the

embryo, and therefore when first formed divided the ovum into an anterior and a posterior half; while the second furrow, the one in which the polar furrow bends to the left, coincides with the median plane of the embryo, and hence divided the first two blastomeres into two right and two left macromeres.[1] While it is thus easy to determine from the earliest appearance of the first cleavage what the antero-posterior axis of the future embryo is to be, it is not possible to distinguish the anterior end from the posterior until the stage with twenty ectoderm cells, Fig. 22, when the mesentoblast is formed. A similar relation of the first cleavage plane to the embryonic axes is also found in Teredo (Hatschek, '80), Umbrella (Heymons, '93), and Nereis (Wilson, '92). In Crepidula I believe it has no causal relation to the bilateral symmetry of the embryo. The egg itself is not bilateral with respect to the first or second cleavage plane, as has been pointed out (p.), but is from the first up to the time when the mesentoblast is formed radially symmetrical. So far as the entoderm cells are concerned, the second furrow lies nearly in the median plane of the bilateral embryo, and the first furrow nearly at right angles to this; but among the ectoderm and mesoderm cells such shiftings of position occur that the final plane of bilateral symmetry in no way corresponds with either of the first two cleavage planes. This conclusion will be treated more fully after the facts upon which it is based have been taken up in their regular order.

II. The Segregation of the Ectoblast.

1. *Formation of the First Quartette of Micromeres. Figs. 12, 13, Diagram 3 (p. 60).*

The third cleavage separates four protoplasmic micromeres from the four yolk-containing macromeres. The karyokinetic

[1] It will be seen that in all the figures except those of the first plate the first furrow runs from right to left on the plate; for the sake of appearance merely, the figures of the first plate are arranged so that the first furrow runs up and down. In the first plate, therefore, the antero-posterior axis runs from right to left as the figures are arranged on the page, while in all the other plates it runs up and down.

spindles which introduce this cleavage have their inner ends at a higher level than the outer ends, and are usually very nearly radial in position, though they are frequently slightly inclined in a right spiral direction, and occasionally even in a left spiral. Whatever may be the direction of the spindles, however, the third cleavage itself is always a dexiotropic one. In the early stages of the formation of these spindles their axes may be radial or even laeotropic; but usually before the nuclear division is completed, and always before the cell division takes place, the cleavage becomes dexiotropic. Thus in Fig. 12 the spindle in the macromere C is most advanced, while those in D, A, and B show progressively earlier stages in the nuclear division. Now if we consider the outer ends of the spindles as remaining stationary, and the inner and upper ends as being movable, it will be seen that in C the inner end has been rotated slightly in a clockwise direction around the chief axis of the ovum as a centre; there may be slight indications of this rotation in D and A, though certainly it is not present in B. In other words, *the more advanced the cleavage is, the more pronounced the rotation becomes*, and what is true in this instance is true in every one that has come under my observation. *After the division wall between the dividing cells has appeared, the rotation still continues; in the formation of both the first and second quartettes there is an actual rotation of these cells*, and not merely an oblique cleavage, as is the case in Unio and in some of the cleavages of Nereis.

In some ova the formation of the micromeres of each quartette takes place in regular succession, as is shown by the successive stages of karyokinesis in the four macromeres of Fig. 12; in other ova no such regular succession can be determined. After the micromeres have been separated they continue to rotate until they come to lie in the furrows between the macromeres and alternate with them in position, Figs. 13 and 14. The outer cell walls of the micromeres are at first rounded, as shown in Fig. 13; but after they have taken their positions between the macromeres they become pressed down into the furrows so that their outer

border becomes pointed, as shown in Fig. 14. *Thus it is seen that the shape of the cell depends, in part at least, upon the position which it holds,* i.e., *the outlines of the cell are the result of the pressure to which it is subjected.* These micromeres at first meet each other in a point immediately under the polar bodies, though afterward, as the result of pressure, two of them may meet in a line or secondary polar furrow, as shown in Fig. 17. This secondary polar furrow is not a part of the original polar furrow, but is a new feature caused by the shifting of the cells of the first group of micromeres after they have been formed. Moreover, it bears no constant relation to the original polar furrow; in Figs. 17, 29, 31, 33, 42, 44, 46 this secondary polar furrow is almost parallel with the original one ; in Figs. 32, 35, 36, 38, 41, 49, 64 it is nearly at right angles to it, and there is evidence that in the same egg it may change its relations at different periods. Among small cells very actively dividing polar furrows, or rather pressure surfaces, do not long preserve definite axial relations. The original polar furrow preserves its fixed position because it lies between macromeres, which in spite of numerous divisions still remain very large ; the position of the polar furrow could not here be changed without profound changes in the positions of all the other cells and in the shape of the whole egg.

In Crepidula the dorsal portion of the polar furrow does not lie between any of the ectoblast cells, since in all cases the cells of the first quartette meet in a point when first formed ; the polar furrow lies wholly and entirely between the macromeres, and its dorsal portion can be seen just beneath the cap of ectoblast cells.

Kofoid ('95) has found that in Limax both the dorsal and ventral polar furrows preserve their identity even up to an advanced stage of the cleavage, and here the axial relations of both furrows are also preserved. Kofoid says (p. 55): "With the completion of the sixteen-cell stage and the fifth generation, the dorsal and ventral cross furrows are restored to the conditions of the four-cell stage, *i.e.*, they cross each other at approximately right angles. A similar restoration to the conditions of the four-cell stage occurs in Nereis; also in Umbrella

at the twelve-cell stage, and probably in Neritina. In Planorbis, however, according to Rabl's interpretation, the cross furrow of the animal pole is not restored to the position of the four-cell stage, but is turned 90° from it (see his Taf. XXXII, Figs. 10 A, 11 A). To accomplish this it is necessary for each of the cells of the apical quartette to be shifted 90° to the left, and thus completely out of their own quadrants over upon the adjoining quadrants. It seems very probable that Rabl is in error in this matter, and that in Planorbis, as in the other forms, the division of the generations results in the restoration of the cross furrows to the conditions of the four-cell stage." It is possible that in Planorbis, as in Crepidula, there is no part of the original polar furrow between the cells of the apical quartette, and that the pressure surface, formed later, may lie in any direction with reference to the real polar furrow.

2. *Formation of the Second Quartette of Micromeres. Figs. 14–16, Diagram 3 (p. 60).*

When the first four micromeres have taken a position alternating with the macromeres, the nuclei of the latter again divide, as shown in Fig. 14. All the nuclei divide at nearly the same time, as in the preceding cleavage, but the spindles do not lie radially as before, but run transversely or tangentially in each macromere. One end of each spindle lies on the mid line of each macromere, the other end lies to the left, very near the furrow, between contiguous spheres; the former is at a lower level than the latter, and hence the spindles are arranged in a left wound or anti-clockwise direction. Again, considering the deeper or central end of each spindle as fixed and the other as movable, it will be seen that as division advances the outer end swings inward toward the centre of the formative pole, and at the same time comes to lie at a considerably higher level, Figs. 14 and 15. As the four cells of the second quartette are being cut off from the macromeres, they rotate in an anti-clockwise direction until they occupy the furrows between the macromeres, and by this rotation they turn the cells of the first quartette back to their original positions over the centre of

each macromere, Diagram 8. These micromeres do not again shift their position to any considerable extent until the general rotation of the ectoblastic cap in the 52-cell stage.

The fact that the micromeres are more firmly bound to each other than to the macromeres is shown hereafter at almost every stage; it is first plainly indicated, however, in such stages as Figs. 15 and 16, where the second quartette of micromeres, in rotating in an anti-clockwise direction, carries with it the first quartette, as if the whole formed a rigid plate lying upon the macromeres. Evidence of this same fact is farther shown by Fig. 16, in which the micromere 2b does not lie in the furrow between A and B, though the other micromeres of this quartette, 2a, 2d, and 2c, lie in the other furrows. This is due to the fact that because of a very long polar furrow between macromeres B and D, the first and second cleavages are not at right angles to each other. Instead, therefore, of shoving past 1a into the furrow between A and B, the cell 2b remains in its proper position relative to the other micromeres, although by so doing it cannot come into the proper position relative to the macromeres.

This fact that the micromeres are more loosely connected with the macromeres than with each other may be in part accounted for by the presence of a small rectangular segmentation cavity lying just over the polar furrow and under the first set of micromeres. It is most clearly marked at the moment when the first quartette is separated from the macromeres, and it entirely disappears after the second quartette is formed.

3. *Division of the First Quartette of Micromeres and Formation of the Turret Cells (Trochoblasts). Figs. 16, 17, Diagram 4 (p. 60).*

Before the third and last quartette of micromeres is formed the first quartette divides in a laeotropic direction, as shown in Fig. 16. Division occurs at nearly the same time in each of the cells, and the central moieties ($1a^1$–$1d^1$) remain considerably larger than the peripheral ones ($1a^2$–$1d^2$). The smaller outer portions do not again divide until very late in the cleavage,

and they therefore form a valuable landmark for orientation. From their peculiar position and shape I shall call them the "turret cells"; their further history will be considered in another place.

After the division of the nuclei, and even after the cell body has divided, the turret cells continue to rotate in a clockwise direction until they lie at the ends of the furrows separating the four apical cells. *In this case, therefore, as in every other which I have observed, the spiral character of the cleavage is much more pronounced after the nuclear division than during that division. It seems to be a phenomenon belonging to and caused by the cytoplasm rather than the nucleus.*

In Discocoelis (Lang, '84), Nereis (Wilson, '92), and Limax (Kofoid, '95) the first quartette divides at the time the second is being formed, and before the third quartette is formed the first has divided twice. In Planorbis (Rabl, '79), Neritina (Blochmann, '81), Unio (Lillie, '95), and Crepidula the first quartette divides once before the third is formed; while in Umbrella (Heymons, '93) and Urosalpinx the first does not divide at all before the third is formed. In general the rate of development of the upper hemisphere is indicated by these facts ; in Nereis the development of the upper hemisphere is very precocious; it is very tardy in Umbrella and Urosalpinx; while Planorbis, Neritina, Unio, and Crepidula occupy an intermediate position in this respect.

In most gasteropods so far studied the turret cells have essentially the same peculiarities of size and position as in Crepidula, so that during the early stages of cleavage they can be recognized at a glance. In Nereis Wilson has found that these cells form the prototroch, and he therefore calls them the *trochoblasts*. Mead ('94) also has found that they form a part of the prototroch in Amphitrite and Clymenella. In Crepidula at least two of these cells, probably all four, form a portion of the velum; but because I am not certain as to the destiny of the two posterior ones ($1c^2$ and $1d^2$), I prefer to call the group for the present by a non-committal name. Their destiny has not been determined in any other form.

4. *Formation of the Third and Last Quartette of Micromeres and Complete Segregation of the Ectoblast. Figs. 17–19, Diagram 4.*

The last quartette of ectomeres is formed by dexiotropic cleavage. The axis of each spindle lies transverse to the median plane of each macromere, and nearer the right side than the left. The right end of the spindle is higher than the left, and lies on the right side of the macromere near the furrow between contiguous spheres, and in the space between successive micromeres of the second quartette. The left and

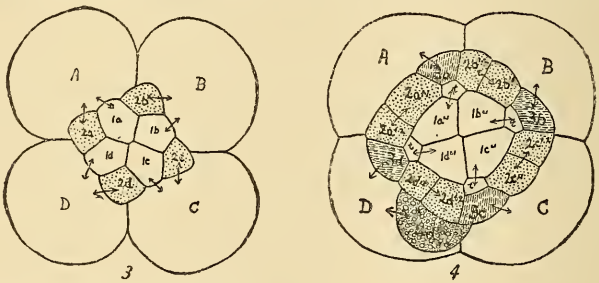


DIAGRAM 3.— Crepidula, twelve-cell stage.

DIAGRAM 4.— Crepidula, twenty-five cells; *t*, turret cells (trochoblasts). In these and some of the following diagrams the macromeres and first quartette are unshaded; the second quartette is stippled; the third quartette is shaded with lines; and the fourth quartette (4d) with dots and circles. The direction of the various cleavages is shown by means of arrows.

lower end of the spindle lies near the mid line of the macromere, and usually beneath the second group of micromeres, Fig. 17. As division advances, the right and upper end of each spindle is lifted to a higher level and swung inward toward the apical pole. When the cells of this quartette are separated they do not rotate in a clockwise direction, carrying the whole plate of micromeres with them, as in the formation of the first and second quartettes, but the ectoblastic plate remains fixed and the third group of micromeres is merely pressed into the spaces between the micromeres of the second group, Figs. 18, 19, 20 and Diagram 4.

Into these three quartettes of micromeres is gathered the entire ectoblast of the developing embryo; and from these

twelve cells (increased to sixteen by the division of the first quartette) comes the whole outer covering of the body, the shell gland and ciliated locomotor apparatus, the larval excretory cells and stomodaeum, the nervous system and sense organs.

The material of the macromeres is not homogeneous as yet, since one of them, the left posterior, contains most of the future mesoblast ; but at this early stage we have two layers, ectoblast and mesentoblast, perfectly differentiated.

It is a most remarkable fact that in all annelid and molluscan eggs with holoblastic segmentation the ectoblast is segregated in just three groups or quartettes of cells — no more and no less. The evidence for this remarkable fact has been accumulating until at present it is known to be true of at least a score of forms, and not a single trustworthy observation can be urged against it. A few apparent exceptions have been recorded among prosobranchiate gasteropods. Bobretzky ('77), after describing the formation of the first two groups of micromeres in Nassa, says : "The large spheres continue to bud off new cells around the circumference of those already formed, while the latter continue to divide." Of Fusus he says that after the first two cleavages the segmentation goes on as in Nassa. Of these statements it need only be said that the work was done at a time (1874–75) when little attention was paid to the details of cleavage, and confessedly the number of quartettes of ectomeres was not known. I have worked over the cleavage of Illyonassa and Urosalpinx, which are the nearest representatives of Nassa accessible to me, and, although the form of cleavage is very similar to that given for Nassa, there is no departure from the rule that three, and only three, quartettes of ectomeres are formed.

Another exception is recorded by McMurrich ('86) for Fulgur. After describing the formation of the first two groups of micromeres, he says : "The succeeding stages of segmentation I did not follow in detail, but can state that they result in the increase of the number of micromeres, partly by the division of those already formed, and partly by the separation of new ones from the macromeres. . . . It would seem that in Fulgur spherules

continue to be budded off from the macromeres for a much longer period than in some other forms ; thus, for instance, Blochmann describes only three generations of spherules from the macromeres in Neritina. In Nassa, according to Bobretzky, a greater number are formed ; he describes twenty spherules as arising in this manner, and it is possible that more arise in the same way in later stages. Probably the amount of yolk present influences the number of spherules so formed ; in other words, the greater the number of spherules required to surround the macromeres, the more frequently are generations formed from the macromeres." Lillie ('95) comments upon this passage, and justly remarks that "the important point is to determine how many of these generations are ectomeres," not all micromeres being ectomeres, as Wilson ('92) has shown in the case of Polymnia and Aricia.

In all four species of the genus Crepidula which I have studied, but three quartettes of micromeres are separated from the macromeres, and this in spite of the fact that the egg of C. adunca is twenty-seven times as large as the egg of C. plana ; in this case, therefore, the size of the egg has no influence on the number of quartettes separated from the macromeres, although a count of the nuclei shows that about five times as many ectoderm cells are present in C. adunca at the time of the closure of the blastopore as are found in C. plana. This increased number of ectoderm cells in the large egg is due entirely to the more rapid division of the three quartettes already formed, and not to the formation of additional quartettes.

McMurrich's conclusions are so much in conflict with my observations on Crepidula that I have taken the pains to briefly study the cleavage of Fulgur. The result of this study shows that in this case also three, and only three, quartettes of micromeres are separated from the macromeres. The cleavage is marvellously like that of Crepidula, though the eggs are from fifty to one hundred and forty times as large.

Two other somewhat doubtful exceptions to this rule have been recorded; *e.g.*, Salensky ('87) believed that more than three quartettes of micromeres were formed in Vermetus. More

recently Erlanger ('92) has reached the same view concerning Bythinia. Neither of these cases, however, is conclusive, and I have little doubt that a careful reëxamination would show that here also three, and only three, quartettes of *ectomeres* are formed.

No phenomenon in the whole history of cleavage seems to me more remarkable than this. As just said, it occurs almost universally among mollusks and annelids, in equal or unequal cleavage, and in eggs varying in size from a few microns to more than a millimeter in diameter. Associated with it is the formation of the mesoblast and entoblast in all these forms in the fourth quartette. *The cause of this remarkable phenomenon is to be found in the fact*, as I believe, *that each of these quartettes of ectomeres is the protoblast of definite regions and organs of the larva.* In all cases in which three quartettes of ectomeres are formed, the first quartette gives rise to all the umbrella region and at least a portion of the prototroch ; the second quartette gives rise to the median anterior, posterior, right and left portions of the body ; while the third quartette gives rise to the regions intermediate between those formed by the second quartette.

In Umbrella the different quartettes are successively larger, the first being smallest and the fourth largest. In Crepidula the difference in size between the first three quartettes is very slight, though the second quartette is perhaps somewhat larger than either the first or third ; the fourth quartette, owing chiefly to the amount of yolk which it contains, is very much larger than either of the preceding ones. In general the relative size of the different quartettes of ectomeres depends upon the relative size of the regions and organs of the larva to which they give rise, and also upon the relative time at which these organs are formed.

5. *Division of the Second Quartette of Micromeres. Figs. 18, 19, Diagram 4.*

Although it is not my purpose to take up the history of the micromeres until after I have described the complete segregation of the layers, it seems best in this section to trace the

history of the whole egg up to the point, Fig. 22, where the segregation of the layers is practically complete, and then to deal separately with the history of each of these layers ; accordingly, I shall describe here the first division of the second quartette, which occurs before the separation of the mesoblast.

Very soon after the formation of the third quartette the second quartette divides, Figs. 18 and 19. It is not possible during the nuclear division to tell which end of the spindle lies at the higher level, though the right end lies nearer the mid line of each macromere, Fig. 19, and after the cell division it is seen that the right moiety overlaps the left, Fig. 20. The spindles are, therefore, arranged in a right-wound spiral, and the division is dexiotropic. The two moieties are about equal in size, though the right one seems the larger because it overlaps to a certain extent the left.

At this stage there are twenty micromeres and four macromeres. The micromeres are arranged in a plate, the rounded corners of which lie in the furrows between the macromeres, Fig. 19, Diagrams 4 and 5. The centre of the plate is formed of four *apical cells* and four *turret cells*, which are the derivatives of the first quartette. These eight cells form a rectangular plate with its corners in the furrows between the macromeres. Around this central plate of eight cells is a belt of twelve cells, consisting of eight cells derived from the second quartette and four cells of the third quartette ; these cells we shall call the *belt cells*. In Fig. 20 it is seen that the apical and turret cells overlap the belt cells, so that the micromeres are arranged like the shingles on a roof. The apical cells do not overlap the turret cells ; in the division of the second quartette, as has been explained, the right moiety overlaps the left; while underlying all of these is the third quartette.

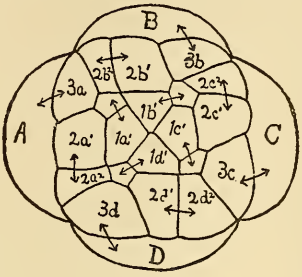
The first division of the second quartette occurs in essentially the same way, though subject to certain variations in time, in all cases in which the cleavage has been carefully studied, with the single exception of Neritina. Blochmann ('81) asserts that in this animal the cleavage is not dexiotropic, as is true elsewhere, but is laeotropic. This difference in itself might seem to be of little importance, but since it profoundly modifies

the interpretation of later stages, it demands a careful consideration.

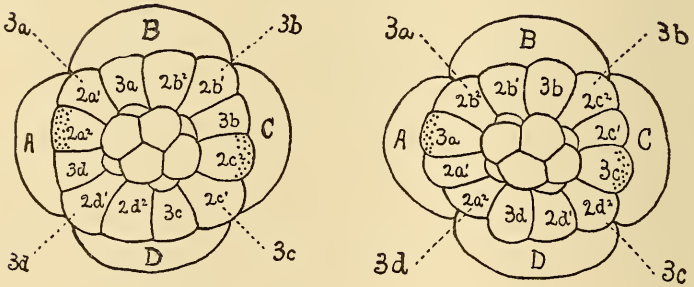
Heymons ('93) called attention to the difference between Neritina and Umbrella in this cleavage, and he ascribed it to the difference in the axial relations of the "cross" of ectoblast cells in those two animals. In this he was certainly in error, as we shall see when we come to consider the cross in a subsequent section.

More recently Kofoid ('94) has discussed this unusual cleavage in Neritina and has presented strong evidence for believing that Blochmann was mistaken in his interpretation of it, and still more recently Lillie ('95) cites Kofoid's criticism with approval. Kofoid suggests a possible correction of Blochmann's interpretation (the nature of which is shown in the accompanying diagram, 5c), which would bring the cleavage of Neritina into conformity with the "law of alternating cleavages," but curiously enough, he just misses the true explanation. The modification suggested by Kofoid does meet the requirements of his law of alternating cleavages, but it does not harmonize with Blochmann's oft-repeated statement that the terminal cells in the transverse arms of the cross (his "Urvelarzellen") come from two cells, 2a and 2c, of the second quartette. Even before cleavage begins two masses of granules can be recognized on opposite sides of the animal pole, and in the formation of the second quartette these granules pass into the cells 2a and 2c, and finally they appear in the "Urvelarzellen," as soon as these are formed. Owing to the presence of these peculiar granules, it seems very improbable that Blochmann could have been mistaken in the derivation of the "Urvelarzellen." Kofoid recognizes this difficulty and attempts to meet it by suggesting that the granules originally present in the cells 2a and 2c may disappear, and that new granules may appear in the corresponding cells of the third quartette (3a and 3c), which are in turn handed over to the "Urvelarzellen." This suggestion seems to me as improbable as it is unnecessary. In Crepidula the terminal cells of the cross ("Urvelarzellen") are derived exactly as Blochmann asserts is the case in Neritina, and the same thing is

true of several other gasteropods which I have studied. I think, therefore, that Blochmann's derivation of these cells can no longer be called in question. But it is evident, as Kofoid points out, that he has made a mistake in the derivation of the



b



a *c*

5

DIAGRAM 5. — The 24-cell stage in Neritina and Crepidula. The nomenclature is the one used throughout this paper. — *a*, The derivation of the belt cells of Neritina according to Blochmann. The stippled cells give rise to the "Urvelarzellen." — *c*, Kofoid's modification of Blochmann's account. — *b*, The derivation of these cells in Crepidula. The reference lines and letters in *a* and *c* indicate the modifications necessary to bring Neritina into agreement with Crepidula.

belt cells. In the accompanying diagrams I give Blochmann's Fig. 48, Kofoid's modification of this, and a corresponding stage in Crepidula; the nomenclature in each case has been reduced to the system used in the present paper.

It will be seen at once that both Blochmann and Kofoid fail to identify the cells of the third quartette with the corners of the ectoblastic plate, and consequently mislabel the whole of the belt, carrying the proper designations one cell too far to the right in the one case, and one cell too far to the left in the other. This proposed correction of Blochmann's account and of Kofoid's modification is further supported by a figure of the egg of Neritina of this same stage, given by Bütschli ('77), Pl. XVII, Fig. 3a, in which the position of the cells plainly shows that the angles of the ectoblastic plate are formed by the third quartette, while the two cells on each side between the angles have evidently come by division from a single cell.

With this slight modification of Blochmann's account Neritina is made to agree in the matter of the belt cells with Nereis, Umbrella, Limax, Unio, four species of Crepidula, Urosalpinx, Fulgur, Sycotypus, and Illyonassa, and at the same time Blochmann's statement as to the derivation of the "Urvelarzellen" is confirmed, and Kofoid's contention for the alternation of cleavages is satisfied.

III. The Segregation of the Mesoblast and Entoblast.

1. *Formation of the Mesentoblast. Figs. 21, 22, Diagram 4 (p. 60).*

At the stage just described, with twenty micromeres and four macromeres, the left posterior macromere divides in a laeotropic direction, as shown in Fig. 21. The cell thus formed is very much larger than any of the micromeres, and, unlike them, contains a considerable quantity of yolk. This cell, although formed by a laeotropic division, remains in nearly the same position in which it was first separated from the macromere until a much later stage, Fig. 33. Like the belt cells it is partly overlapped by the micromeres which lie nearer the apical pole, but a considerable part of it is exposed on the surface. In a strict use of the term, therefore, it cannot be said at this stage to form the *middle layer* any more than the belt cells form a middle layer. In fact, it is neither a "layer" nor is it "middle," and yet from a part of this cell most of the

mesoblast is developed. Since this cell gives rise to the posterior part of the alimentary canal as well as to the mesoblast, I shall call it the *mesentoblast*, ME (= 4d).

Soon after its formation it divides, as shown in Fig. 25, into right and left halves, ME^1 and ME^2; this division is dexiotropic, as is shown by the fact that the right half overlaps the left, Figs. 26 and 27. These cells remain for some time in the position in which they are formed; they lie to the right of the future median plane, which is marked by the second cleavage furrow, and are more nearly symmetrical with reference to the ectoblastic plate than to the macromeres, Figs. 26, 29, 30. The next cleavage of these cells, Fig. 30, leads to the formation of

2. *The Primary Enteroblasts. Figs. 30, 31.*

The spindles which introduce this division are bilaterally symmetrical with reference to the line along which the two cells are in contact; anteriorly the spindles diverge from this line and at the same time slant upward, so that the cells which are given off anteriorly lie at a higher level than the posterior moieties, Fig. 31. These anterior cells are about equal in size to the posterior ones, but contain less yolk. The posterior cells are the *primary enteroblasts*, and together with two other cells, to be described in a moment, give rise to the posterior or distal end of the intestine. They are purely entoblastic, and do not divide again until about the time of the closure of the blastopore. The anterior cells are still of mixed character, containing both mesoblast and entoblast.

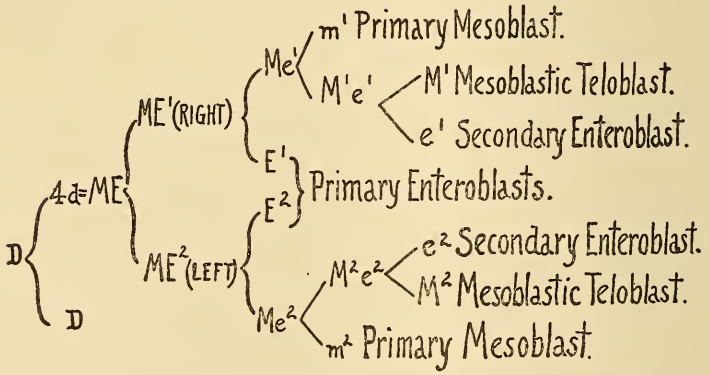
Up to the last cleavage there had not been a single bilateral division; even in the formation of the mesentoblast and its division into right and left halves, all the cleavages were spiral. But with the division of the right and left mesentoblasts, by which the primary enteroblasts are cut off posteriorly, bilateral cleavages suddenly appear. All subsequent divisions of the mesoblast, as far as I have been able to follow them, are bilateral. In the ectoblast and entoblast, however, bilaterality appears very gradually, and is not prominent until a very late period.

3. *The Primary Mesoblasts. Figs. 32–39.*

The two anterior cells resulting from the preceding division are still mesentoblasts, and the mesoblastic and entoblastic substances in these cells are not completely separated until after two more purely bilateral divisions. The first of these divisions, Fig. 32, occurs immediately after the formation of the primary enteroblasts, and gives rise to two small cells, m^1 and m^2, Figs. 33, 35, 36, which are the *primary mesoblasts*, *i.e.*, they are the first purely mesoblastic cells formed. They are not, however, strange as it may seem, the "pole cells" of the mesoblast; they or their derivatives form the *anterior* or distal end of the mesoblastic bands, and not the *posterior*, growing end. The two posterior products of this division, M^1e^1 and M^2e^2, still contain both mesoblast and entoblast. Finally, by another division of these two cells, Fig. 41, the mesoblast and entoblast are completely separated. This division is also purely bilateral, and results in the formation of the *mesoblastic teloblasts*, M^1 and M^2, Figs. 42 *et seq.*, and the *secondary enteroblasts*, e^1 and e^2. The latter cells lie in front of and overlap the primary enteroblasts, E^1 and E^2, and like these they are in contact with each other along the mid line. The mesoblastic teloblasts lie laterally to the secondary enteroblasts, and are far removed from each other, Figs. 42 *et seq.* Counting from the formation of the mesentoblast, 4d (the primary mesoblast of most authors), it has taken eight cell divisions to bring about the complete segregation of the mesoblast and entoblast in this region of the egg. This can be seen at a glance in the table of the lineage of 4d on the following page.

It is at once apparent from this table that there is a very intimate connection, at least in origin, between the mesoblast and the entoblast. The cell 4d seems to contain all the mesoblastic substance which was originally present in the macromere D; but it also contains a considerable amount of entoblastic substance, less than half the cell being destined to form mesoblast. The separation of the mesoblastic and entoblastic substances in this cell begins by the formation of the primary enteroblasts, E^1 and E^2, on the posterior side of the

two cells into which 4d divides, and is further continued by the separation of the primary mesoblasts, m^1 and m^2, on the anterior side. At this stage there are three cells on each side derived from 4d : the primary enteroblast behind, the primary mesoblast in front, and a mesentoblast cell in the middle, Figs. 33–41. Finally the segregation is completed by the division of the middle cell of the three into a secondary enteroblast behind, and a mesoblastic teloblast in front, Fig. 41. This final separation of the mesoblast from the entoblast does not occur until there are sixty-five cells present, of which eight cells are the progeny of 4d. Of these eight cells four are entero-



blasts and four are mesoblasts, and the latter are almost immediately increased to six by the division of the two primary mesoblasts, m^1 and m^2, Fig. 42. The mesoblast cells form a short band, one on each side, which extends forward almost parallel with the edge of the ectoblastic plate, but entirely covered by ectoblast cells ; the enteroblasts are but partially covered by ectoblast until a relatively late stage. The mesoblast cells are further characterized by containing no yolk, while both pairs of enteroblasts contain a considerable number of yolk spherules.

This method of the separation of the mesoblast is, I believe, unique, and I should be inclined on that account to doubt the correctness of the description here given were it not for the fact that I have followed the lineage of the cell 4d with the

greatest care throughout the stages shown in the table above, and in Figs. 22–41 in the plates; and beyond this stage I have traced the enteroblasts and the mesoblastic teloblasts step by step, until the latter give rise to the mesoblastic bands extending half way around the egg, Figs. 49, 51, 53, and the former apparently become the distal portion of the intestine, Figs. 61, 65, 68.

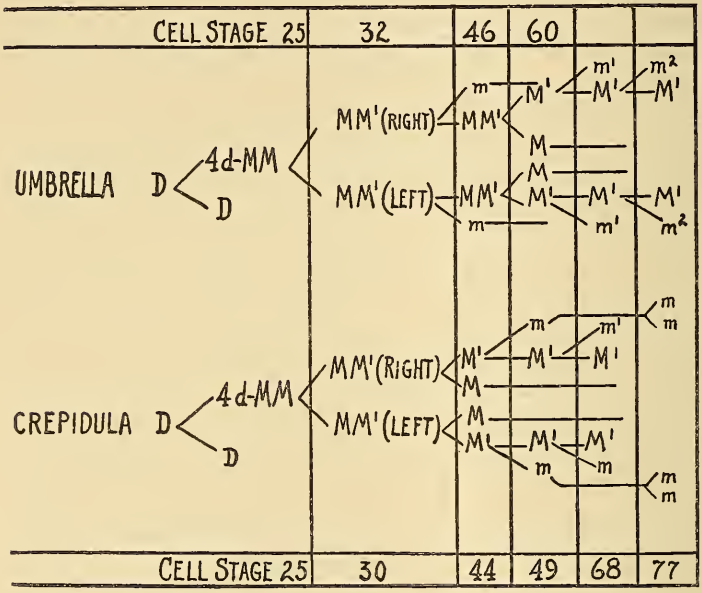
Owing to difficulty in tracing these cells I was not able to work out their history satisfactorily for a long time. Consequently in both of my previous papers on this subject ('91 and '92) I described the cell 4d as the primary mesoblast, feeling assured that it was this because it gave rise to mesoblastic bands. It was not until I had taken up the later history of the entoblast and the formation of the alimentary canal, that I found that the two proximal (originally posterior) cells in each band were the first intestinal cells, and that therefore the cell 4d contained both mesoblast and entoblast.

So far as I know, but two other cases at all similar to this are said to occur among Mollusca. In Patella, Patten ('86) has described two "entomesoblast" cells, which lie one on each side of the four large cells at the vegetal pole. These large cells are entoblastic, and probably correspond to the four macromeres present in many other forms. The entomesoblasts appear at the blastula stage, and after elongating into the cavity of the blastula, each cuts off a large cell at its inner end, which is the mesoblastic teloblast; the outer part of each cell is entoblast.

The other case is given by Stauffacher ('93) for Cyclas cornea. In this animal the formation of the mesoblast is similar to the process in Patella, except that the two mesentoblasts are in contact along the mid line as they are in Crepidula, whereas they are said to be separated by the four macromeres in Patella. In both these cases, however, the resemblance to Crepidula extends no farther than the formation of paired mesentoblast cells, and even in this regard the resemblance is more apparent than real.

In the formation and subsequent divisions of the cell 4d, Umbrella is strikingly like Crepidula. This resemblance is

briefly indicated by the following tabular comparison of the lineage of 4d in these two animals. To facilitate comparison I use throughout the same designations for Crepidula and Umbrella.



Heymons' figures indicate that the resemblance is much closer than the above-given table would show, *e.g.*, all the cells designated by capitals are large cells containing a considerable quantity of yolk; they are about equal in size, are grouped in a characteristic way, and in every respect resemble the enteroblasts of Crepidula. Again, the second division of 4d is equal in Crepidula, unequal in Umbrella; the third division is unequal in Crepidula, equal in Umbrella. The time relations of these two divisions are simply reversed.

Although the formation and subsequent divisions of the cell 4d are so similar in Crepidula and Umbrella, my interpretation of the destiny of some of the cells derived from this blastomere is wholly different from that given by Heymons. According to this author, the entire cell 4d and all of its derivatives are purely mesoblastic. Heymons has followed all of the divisions

of 4d up to a very late stage, and with such accuracy of detail that one can scarcely doubt the soundness of his conclusions. In fact, his results have led me seriously to doubt the correctness of my own interpretations ; but after a careful reëxamination of my preparations and drawings, I am unable to reach any other conclusion than that already given. I cannot say that I have actually observed the cells which I have called the enteroblasts going into the formation of the intestine. The last stage in which they could be identified beyond any doubt is shown in Fig. 65, and between this stage and those shown in Figs. 68 and 76 there is more or less discontinuity. Still I believe that the evidence is clearly in favor of the view which I have advanced. The enteroblasts E^1,E^2,e^1,e^2 are not only in exactly the right position to form the distal end of the intestine, but they differ in histological character from the mesoblast cells, and closely resemble the entoblasts in that they contain a number of yolk spherules; besides in all the later stages the large cells which I have called the mesoblastic teloblasts, M^1 and M^2, are plainly visible lying at the posterior ends of the rather indistinct mesoblastic bands. These cells are frequently seen dividing, but during all the time that the mesoblastic bands are forming, the enteroblasts never divide, which is, I think, pretty good evidence that they are not the mesoblastic teloblasts.

In Heymons' figures of the later stages (see his Figs. 29 and 30), the position and appearance of the mesoblastic teloblasts is so similar to the corresponding cells in Crepidula, that one can scarcely doubt that they are homologous cells. However, between the two teloblasts in Umbrella is a group of small cells, which, from their position, should correspond to the enteroblasts of Crepidula, but which seem to be very different in origin and history from these cells. Of this group of cells Heymons says, p. 281 : "Zwischen den beiden aus einander gewichenen Urmesodermzellen befindet sich eine Anzahl von 4–6 kleineren Mesodermzellen. Dieselben entsprechen der Zellgruppe, an deren Bildung die zuerst entstandenen kleinen Mesodermzellen *m m* Antheil nahmen. Letztere sind jetzt allerdings nicht mehr als solche herauszufinden.

Die erwahnte Zellgruppe liegt in der Medianlinie am Hinterende der Schalendrüseneinstülpung." In short, this group of cells lying between the teloblasts in Umbrella, corresponds in origin to the most anterior derivatives of 4d in Crepidula, which ultimately lie at the anterior ends of the mesoblastic bands; the cells which lie between the teloblasts in Crepidula are the most posterior derivatives of 4d.

Von Wistinghausen ('91), Wilson ('92), and Lillie have observed that a number of small cells are budded off on the surface of the primary mesoblasts in Nereis and Unio. Wilson says that these small cells later wander into the cleavage cavity and form "secondary mesoblast," and Lillie believes that the same thing happens in Unio. It is to be observed that the enteroblasts of Crepidula are, for a long time, uncovered by the ectoblast cells, and that they apparently lie in the layer of ectoblast, and in this regard resemble the small cells described by the authors just mentioned. It is scarcely possible that those small cells are homologous with the enteroblasts in Crepidula, but it is sufficiently obvious that in many cases the history of the so-called "primary mesoblast" has not been followed far enough to determine whether it gives rise to anything else besides mesoblast. If a cell arises in the proper place on the posterior side of an egg, and gives rise to a row or band of cells, it is generally supposed to be sufficient ground for calling it the primary mesoblast. I believe that the so-called "primary mesoblast" of many other gasteropods would be found to contain both entoblast and mesoblast if its later history were carefully followed.[1]

In Planorbis and Umbrella the cell 4d arises at the 24-cell stage, as it does in Crepidula; in Unio it appears when 32 cells are present; in Neritina at the 36-cell stage; in Nereis at the 38-cell stage; while in Limax it is not separated until the 64-cell stage. This apparent difference in the time of its formation is due chiefly to the fact that in some cases the ecto-

[1] Blochmann's Figs. 62 and 63 for Neritina show two mesoblast bands of two cells each, and between them anteriorly a number of small entoblast cells, some of them closely connected with the mesoblast. These entoblast cells are of doubtful origin, and it may be that they correspond to the enteroblast cells of Crepidula.

meres divide more rapidly than in others. But in all these cases the cell 4d is formed in the fourth quartette of cells separated from the macromeres. The essential likeness in origin of this cell in all these forms is thus clearly shown, though it arises at apparently different times in different eggs.

In addition to the mesoblast thus formed, which is bilateral and teloblastic in growth, three other mesoblast cells arise from the ectoblast in Crepidula at a much later stage. These cells, which correspond to the "larval mesoblast" of Unio (Lillie ('93), p. 570), appear in the quadrants A, C, and B, and give rise to the scattered mesoblast cells in the region of the blastopore, and at the anterior end of the embryo. The origin of these cells cannot be described satisfactorily until the later history of the ectoblast has been considered (see p. 149).

Although I do not propose in this section to take up the history of the different layers, yet it seems best here to describe the complete separation of the fourth quartette of cells to which 4d belongs.

4. *Completion of the Fourth Quartette and Rotation of the Ectoblast. Figs. 33, 34.*

A laeotropic division in the 24-cell stage separated the mesentoblast from the left posterior macromere; the corresponding divisions in the other macromeres are delayed until the stage with 49 cells, Figs. 33, 34. At this stage each of the macromeres, except the left posterior one, gives rise to a large yolk cell, 4a, 4b, and 4c, by a laeotropic division. The cells thus formed are a little larger and contain much more yolk than the mesentoblast 4d. They move around into the furrows between the macromeres, and ultimately take part in forming the ventral wall of the mesenteron.

No divisions corresponding to this are given for Nereis or Unio.[1] In Limax and Planorbis the cells 4a, 4b, 4c are separated at the same time with 4d; in Umbrella at about the same stage as in Crepidula; in Neritina two cells which seem to cor-

[1] Mead ('94) briefly mentions the fact that these divisions occur in Amphitrite, Clymenella, and Lepidonotus.

respond to 4a and 4c are formed almost immediately after 4d, and before the latter divides into right and left halves. At a later stage two other cells, designated by Blochmann en_a and $en_a{}^1$, are supposed to have come from the macromere *a* (B of our system). In Blochmann's figures they are shown in the segmentation cavity, half way between the animal and vegetal poles. All of these cells, together with en_x, a small entoblast cell whose origin was not known, are shown in the figures[1] moving up through the space between the macromeres into the segmentation cavity on the upper side of the egg. It seems a very remarkable thing that entoblast cells should travel through the segmentation cavity in this way. So far as I know, nothing like it occurs in any other animal, and I find it hard to believe that Blochmann is right on this point. There are too many points of agreement between Crepidula and Neritina throughout the entire development to make probable the view that they are so wholly unlike in this one regard. Only one figure of the small entoblast cells given by Blochmann has a familiar appearance, and that is his Fig. 64, in which three small entoblast cells are shown at the vegetal pole in the positions occupied by 4a, 4b, and 4c in Crepidula.

These fourth-quartette entomeres were observed and figured by McMurrich ('86) for Fulgur, though he did not suspect their real nature. He says of them (p. 413): "On surface view three elongated elevations (Plate XXIV, Fig. 8) are seen radiating toward the centre of the blastodermic area, but not extending centrally farther than the edge of the area, and lying rather alternate with the macromeres than opposite them. What the significance of these elevations may be it is not easy to say, but sections through ova of this stage show them to be coincident with the first formation of the *mesoderm*. . . . If this interpretation of the sections be correct, it would seem that the macromere which does not show an elevation on surface view is the one which gives rise to the mesoderm, but what may be the cause of the formation of the elevations on the macro-

[1] In interpreting Blochmann's account of these smaller entoblast cells I have been compelled to rely largely upon his figures, since little mention is made of them in the text.

meres is to me quite uncertain. I think it safe to conclude that the mesoderm arises by a separation of protoplasm from *one* of the macromeres." I find that these elevations are the small yolk cells 4a, 4b, and 4c. They appear somewhat later than the cell 4d, just as is the case in Crepidula.

That these cells really belong to the same quartette as 4d is shown not only by their position and method of origin, but also by the fact that the macromere D does not divide again until a very late stage, and then in a series of divisions which affects each of the other macromeres and leads to the separation of a fifth quartette (5a–5d) from the macromeres. And that all the cells of the fourth quartette, like those of every other quartette, are really homodynamous, is strongly suggested by the fact that they are all yolk cells of about the same size, that they are chiefly entoblastic (4a, 4b, 4c entirely so, and 4d more than half), and that the points in which 4d differs from the other members of this quartette are probably due to the posterior elongation of the body and the origin of bilateral symmetry.[1]

Since these fourth-quartette entomeres are smaller than any other cells of the inner layer except the enteroblasts, I shall call them the *smaller enteroblasts*. Like the mesentoblast, 4d, they are formed by a laeotropic division, and immediately after they are separated they begin to rotate in an anti-clockwise direction, until they come to lie in the furrows between the macromeres, and in this position they are carried around to the ventral side with the growth of the ectoblastic cap. At the same time that these cells rotate to the left all the derivatives of 4d also rotate in the same direction, and thus come to lie at the posterior end of the second furrow; and what is more remarkable, the whole ectoblastic cap is rotated with these cells through almost 45°. There is here furnished another evidence

[1] In a previous paper ('92) I called attention to the fact that the cell 4d is homodynamous with the other cells, 4a, 4b, and 4c of the fourth quartette. Heymons ('93), who reached the same conclusions in his work on Umbrella, curiously misinterprets me on this point. He says (p. 270): "Nach Conklin sollen dagegen die primaren Darmzellen (the macromeres A, B, C, and D) in Ursprung und Lage den beiden Urmesodermzellen entsprechen, eine Ansicht, die ich für Umbrella entschieden zurückweisen muss." A reference to my paper will show that I there advanced exactly the view which was afterwards advocated by Heymons, and is still further elaborated in this paper.

that the micromeres are more firmly bound to each other than to the macromeres, and the explanation of this fact cannot be found in this case in the presence of a segmentation cavity, since this cavity has long before completely disappeared. Although it has not been mentioned before, it will be seen by consulting the figures that before this general rotation of the upper pole takes place (Fig. 33) the ectoblast on the posterior side of the egg has become bilaterally symmetrical. There is formed at the upper pole, as will be described later, a cross of ectoblast cells, the four arms of which lie nearly half way between the first and second furrows, and hence in the median planes of the macromeres. These arms at first consist of two cells each, Figs. 29, 30, but in the stage represented in Fig. 31, in all the arms except one the number of cells is increased to three; this one, which lies over the left posterior macromere, contains for a very considerable period only two cells. This is one of the first traces of a bilateral arrangement of the micromeres, though it soon becomes very well marked. In C. adunca bilateral symmetry does not appear in the ectoblast until still later, three cells being formed in the posterior arm of the cross as in each of the others. In the egg of this species, in which the larval history is most completely suppressed, and which might, therefore, be supposed to have adult characters impressed upon it at an earlier period than in eggs with a larval development, bilaterality appears later than in either of the other species.

Almost from the earliest appearance of the mesentoblast it is in itself bilaterally symmetrical. The divisions which lead to the formation of the primary enteroblasts and the primary mesoblasts are, as we have seen, typically bilateral, and their plane of symmetry very nearly coincides with that of the ectoblast cells.

Although the macromeres have from the first been radially symmetrical, as is shown by the presence of the polar furrow, yet the future plane of bilateral symmetry is well marked in them, since the first and second cleavage planes which separate them lie respectively in the transverse and median planes of the embryo; the plane of bilateral symmetry in the entoblast lies at an angle of nearly 45° with that of the ectoblast and

mesoblast, and it is not until the three smaller entoblasts are formed and the whole of the ectoblast has been rotated in an anti-clockwise direction that the planes of symmetry in the three layers come to coincide in the median plane of the future animal.[1] The bilateral symmetry which is to characterize the adult appears at different times and in different directions in each of the layers, and at a later period these planes, which have been diversely established, come to coincide in the chief axis of the developing organism. No better evidence could be desired to show that such forms of cleavage are coenogenetic, and that at the same time they are not the result of merely mechanical causes. *In cleavage, as in the entire ontogeny, one is impressed with the evident purposefulness of every event; the end seems to be in view from the beginning, and the building materials are sorted and arranged with reference to this end result.*

In this connection it is interesting to inquire into the causes which produced this rotation. There can be little doubt that it is due to the three smaller entoblasts, since at this time there is no apparent activity in any other part of the ovum. These cells are given off in a left spiral cleavage, and they lift the overlying ectoblast cells and turn them in an anti-clockwise direction until these three entoblasts lie in the furrows between the macromeres, and so the egg is left in as compact a form as possible.

Heymons ('93) has shown that an exactly similar rotation of the ectoblast takes place in Umbrella. The rotation occurs at the same time, in the same direction, and to the same extent as in Crepidula. Heymons also assigns the same cause which I have attributed both here and in my former paper ('92), *viz.*, the rotation of the small entoblasts into the furrows between the macromeres.

Fig. 33 seems to indicate that the primary enteroblasts, E' and E^2, were prevented from rotating into the furrow between D and C by the pressure of the overlying cells, for as soon as the latter are lifted by the formation of the smaller entoblasts

[1] It is not strictly true that the planes of symmetry in the three layers coincide after the rotation of the fourth quartette, though they are brought much nearer together by that rotation. Even in the stages immediately preceding the formation of the larva, Figs. 65–76, it can be seen that the apical cells of the ectoderm, *Ap.*, still lie to the right of the median plane in the entoderm.

the enteroblasts rotate into this position in advance of the ectoblastic cap. In such eggs as the one shown in Fig. 34 it is seen that the entoblastic derivatives of the cell 4d have rotated farther in an anti-clockwise direction than the overlying mesoblast and ectoblast. Although the cell 4d was formed at a much earlier period than the corresponding cells 4a, 4b, and 4c, it does not rotate until the latter cells are formed, when all rotate together, but at first only the lower or entoblastic derivatives of 4d join in this rotation. In this process the ectoblast is wholly passive, and the rotation of the smaller entoblasts seems to be the result of purely mechanical causes (*e.g.*, surface tension and consequent intercellular pressure); and yet these mechanical causes are governed and directed by the higher coördinating forces which are at work in the building of the organism. For example, the mechanical conditions would have been perfectly satisfied if the smaller entoblasts had rotated in the opposite direction and had carried the ectoblastic cap with them, so that as a result the planes of symmetry in the ectoblast and entoblast would not have coincided, but would have crossed each other at right angles. *We must find the ultimate cause of this anti-clockwise rotation not in such external mechanical conditions, which are, however, incidentally fulfilled, but in those more complex internal conditions, which direct the course of ontogeny, and which in our ignorance we call the coördinating force, or hereditary tendency.*

5. *The Four Macromeres, or Basal Quartette. Figs. 34, 37, 42, 52.*

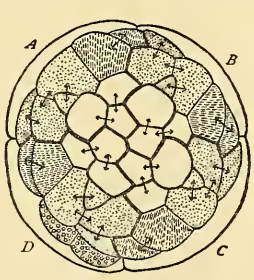
At the time when the layers are all segregated the macromeres still form much the largest part of the egg. They are composed almost entirely of yolk, and their nuclei and protoplasmic portions lie near the surface just in advance of the ectoblastic cap. The four cells are nearly equal in size, and they are from this stage onward closely pressed together, so that the egg is nearly spherical in form and never again assumes the quatrefoil shape. The polar furrow extends between these cells from the vegetal to the animal pole, though on the upper side of the egg it is covered by the cap of ecto-

blast cells. After the formation of the fourth quartette the macromeres do not divide again until a little before the closure of the blastopore, Fig. 54, and consequently the first and second cleavage planes and the polar furrow serve as excellent landmarks throughout a period when the egg is becoming confusingly complex.

In closing this section on the segregation of the layers it may be well to summarize the number and position of the cells in the three layers at the stage shown in Fig. 33.

1st Quartette, Ectoblast Cells		15
2d " " "		16
3d " " "		8
		39
4th "	Primary Mesoblasts	2
	Mesentoblasts	2
	Primary Enteroblasts	2
	Smaller Entoblasts	3
		9
Macromeres, Larger Entoblasts		4
	Total	52

But for the lack of one cell in the posterior arm of the cross there would be ten ectoblast cells in each quadrant, and this layer would be radially symmetrical. In C. adunca, as



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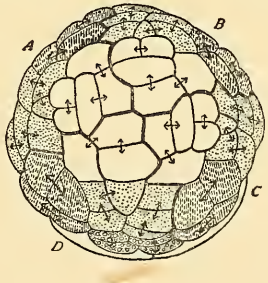

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DIAGRAM 6. — Forty-two cell stage of Crepidula. Shading as in Diagrams 3 and 4. The cross (shown in strong outline) lies in the position in which it was first formed. The heavy, radiating lines separate the cells of the different quadrants.

DIAGRAM 7. — Sixty-cell stage of Crepidula. Shading and heavy lines as in the preceding. The whole of the ectoblast has rotated to the left, due to the rotation of the fourth-quartette cells. The " middle cells " in three arms of the cross have divided transversely. The third-quartette cells on the posterior side have divided bilaterally.

has been said, this cell, which is lacking at this stage in the other species, is present, and the ectoblast is radially symmetrical. The four macromeres may still be considered radially symmetrical. The cells of the fourth quartette lie on the sides of the egg in the furrows between the four macromeres, but the radial symmetry of the egg is destroyed by the behavior of the posterior member of this quartette, 4d. All the divisions of this cell except the first one are purely bilateral in position.

IV. History of the First Quartette of Ectomeres. Diagrams 6–11.

Owing to the presence of certain peculiar landmarks, I have been able to follow the cell lineage of the first quartette farther than that of the others. The derivatives of this first quartette give rise to the whole apical region of the embryo, *viz.*, all the ectodermal cells of the head vesicle, an apical plate of ciliated cells, the posterior cell plate, the dorsal portion of the functional velum and a portion of the first velar row on the ventral side, the supraoesophageal ganglia and commissure, the cerebro-pedal connectives, and, possibly, the pedal ganglia, an apical sense organ, and the paired eyes.

Wilson ('92) has shown that in Nereis limbata and N. megalops this quartette gives rise not only to the entire upper hemisphere of the trochophore, but also to the head kidneys and all the cells of the prototroch. This is not the case in Crepidula ; all that portion of the velum which lies at the ends of the right, the left, and the anterior arms of the cross being derived from the second quartette, while only the intermediate portions come from the first quartette. The anterior branch of the velum on the dorsal side of the body is also derived from the first quartette. As to the head kidney,[1] it is not present in marine prosobranchs, as is well known.

The first division of the cells composing this first quartette gives rise to the *turret cells*, as has been described (p. 58). Their second division occurs immediately after the formation of the mesentoblast 4d, as shown in Fig. 23 ; each of the four

[1] Wilson has lately suggested that this structure may be a mucous gland as Mead found to be the case in Amphitrite.

cells divides in a dexiotropic direction, and in a plane at right angles to that of the previous cleavage. When the division is completed it can be seen that the outer daughter cells are largely overlapped by the apical or stem ($1a^{1.1}$–$1d^{1.1}$) cells. The outer cells ($1a^{1.2}$–$1d^{1.2}$) become the *basal cells* in the arms of a cross, the origin, history, and significance of which will now be considered.

1. *The Ectoblastic Cross.*

In the history of this ectoblastic cross, and of the four turret cells which lie between its arms, is comprised the history of the whole upper hemisphere of the larva. It is the one landmark which has made it possible to follow the cell lineage in some cases to the formation of definitive organs. In treating, then, the history of the first quartette, I shall first deal with the cross, and then take up the turret cells.

(a) *Formation.* — Although two-thirds of all the cells entering into the cross are present and in position at the stages shown in Figs. 23–25, the cross itself does not become apparent until the four small cells have been formed which become the *tip cells* of its arms, Fig. 29, $2a^{1.1}$–$2d^{1.1}$, and Diagram 6. These tip cells come from the second quartette, though all the other cells of the cross belong to the first; for convenience, however, I shall here treat of the cross as a whole, though its tip cells would properly come under the section on the history of the second quartette of ectomeres. The tip cells are formed by an oblique and unequal division of four of the *belt cells*, $2a^1$–$2d^1$, the cleavage being distinctly laeotropic. The upper and smaller moiety becomes the terminal or tip cell ($2a^{1.1}$–$2d^{1.1}$) in each arm of the cross, though its relation to the other cross cells is so close that I doubt whether any one who had not watched its formation would suspect that it was not derived from the basal cells of that structure. The cross then contains all the cells of the first quartette except the turret cells, and in addition the tip cells, which come from the second quartette. When it first appears it consists of twelve cells; the four apical cells form its centre, while there are two cells in each arm, one basal, the other

terminal ; the basal cells were produced by the second division of the apical cells (the turret cells were formed at their first division), and, as just explained, the terminal cells are derived from the second group of micromeres. (See Diagram 6.)

(b) *Axial Relations.* — When first formed the centre of the cross lies exactly at the animal pole of the egg, and the polar bodies are attached at the point where the four apical cells meet. The arms of the cross lie between the first and second cleavage furrows, about 30° or 40° to their right, *i.e.*, in a clockwise direction from those furrows, Figs. 29–32.

At the period when the three smaller entoblasts are formed, Fig. 33 and Diagram 7, the whole ectoblastic cap is rotated to the left until the arms of the cross come to lie nearly over those furrows, so that one arm is approximately anterior, one posterior, one right, and one left. This is not strictly true, since it can be seen by consulting the figures that even after the general rotation of the ectoblast shown in Fig. 33 the arms of the cross do not lie in the furrows between the macromeres, but slightly to the right of them. This continues to be true up to a late period in the cleavage, *e.g.*, in Figs. 51 and 53, the left arm of the cross is distinctly farther forward than the right, while in these figures and a great many others, *e.g.*, Figs. 64, 65, 68, 71, 72, 75, and 76, the anterior arm, which has now grown around to the ventral side, lies to the right of the mid line of the embryo. Ultimately, however, the anterior arm, which can be much more easily followed than the others, comes to lie precisely in the median plane, Figs. 79, 81, 82.

By another and much greater shifting of the ectoblast, which will be described in another section, the entire cap of ectoblast is carried forward through an angle of about 90°. This forward shifting goes on at the same time that the ectoblast is rotating in an anti-clockwise direction, so that by the time that the anterior and posterior arms lie in the median or second furrow, which can still be plainly seen between the yolk cells, the transverse arms lie anterior to the transverse or first furrow.

From its earliest formation up to a late stage in its history the cross in itself is distinctly dexiotropic ; *i.e.*, each arm taken in connection with the apical cell from which it is chiefly

derived forms a linear series of cells, the apical one of which lies to the right of the apical pole. The four arms thus form a right-wound spiral around the apical pole. This arrangement is especially noticeable in the nuclei of these cells, and can here be recognized at the first glance. After the longitudinal splitting of the arms and the division of the apical cells to form the "rosette," this dexiotropic arrangement of the arms of the cross can no longer be recognized.

(c) *Later History.* — Starting from the earliest appearance of the cross, when it contains twelve cells, the cell lineage of the entire structure has been followed to a stage when it contains sixty-six cells, Figs. 53, 56, and Diagram 11. The first cells of the cross to divide are the basal cells in the anterior, the right and the left arms ($1a^{1.2}$, $1b^{1.2}$, $1c^{1.2}$) in the 44-cell stage, Fig. 31. In all the species save C. adunca the division in the basal cell of the posterior arm is delayed until a considerably later period, Fig. 42. By this division, which is slightly dexiotropic, the basal cells are divided into a larger peripheral moiety, the *middle cell* ($1a^{1.2.2}$–$1d^{1.2.2}$), and a smaller apical one, still called the *basal cell* ($1a^{1.2.1}$–$1d^{1.2.1}$). Each arm save the posterior contains at this stage (Figs. 32, 35, 36) three cells, — a basal, middle, and terminal.

When there are 66 cells present, Fig. 42, the basal and terminal cells of the posterior arm divide, the spindle in each case being parallel to the long axis of the arm. In this way the posterior arm comes to be composed of four cells arranged in a linear series, the two proximal ($1d^{1.2.1}$ and $1d^{1.2.2}$) derived from the basal cell and the two distal ($2d^{1.1.1}$ and $2d^{1.1.2}$) from the terminal one, Diagram 8.

About the same time that the cells of the posterior arm divide radially, the middle cell in each of the other arms divides in a plane nearly transverse to its long axis into two equal portions, the *left and right middle cells*, $1a^{1.2.2.1}$ and $1a^{1.2.2.2}$, $1b^{1.2.2.1}$ and $1b^{1.2.2.2}$, etc., Fig. 42 and Diagram 7. The left moiety in each arm is a little nearer the apical pole than the right, and the cleavage is therefore laeotropic. This division of the middle cell in the anterior, the right, and the left arms is the beginning of a longitudinal cleavage of each of these arms,

which is continued until, as shown in Fig. 49, they are split from base to tip.

Before this longitudinal division of these three arms is completed, the four central or apical cells divide in a laeotropic direction ; by this division four central and four peripheral cells are formed. The former ($1a^{1.1.1}$–$1d^{1.1.1}$) are the *apical rosettes* (Wilson ('92), p. 392); the latter are the *peripheral rosettes* ($1a^{1.1.2}$–$1d^{1.1.2}$). The peripheral rosettes are slightly larger than the apical cells, and lie just central to the turret cells and between the basal cells of adjacent arms, Figs. 44, 45. The division of the four apical cells ($1a^{1.1}$–$1d^{1.1}$) is rarely simultaneous, and yet the sequence of cleavage follows no invariable order. In the ova figured the cells of the second and fourth quadrant have divided, while those of the first and third are just dividing.

At the same time that the apical cells are dividing, the terminal cell of each arm, except the posterior, divides into two small cells. This division is frequently very irregular ; in Figs. 44 and 45 it is dexiotropic in the right and anterior arms, and laeotropic in the left ; in other words, the cleavage is bilateral in the transverse arms. This is, I think, the most frequent condition, but there are many deviations from this form. The products of this division are the *right* and *left tip cells*. Finally, the longitudinal splitting of all the arms, except the posterior, is completed by the equal division of the basal cells (Figs. 46, 47, and Diagram 8) into right and left portions, the *right* and *left basals* ($1a^{1.2.1.1}$ and $1a^{1.2.1.2}$, $1b^{1.2.1.1}$ and $1b^{1.2.1.2}$, etc.). This division is very nearly meridional, but subsequent stages, *e.g.*, Fig. 50, show that the left moiety is a little nearer the apical pole than the right ; the division is, therefore, laeotropic.

The cross now consists of 30 cells, as follows :

Apical cells	4
Peripheral rosettes	4
Post. arm — 1 basal, 1 middle, 2 terminal	4
Ant. right and left arms, each 2 basal, 2 middle, 2 terminal cells, 6 in each arm. In 3 arms	18
Total	30

These cells all belong to the first quartette except the two terminal cells of each arm, which were derived from the second quartette, Diagram 8. The cells in three of the arms, the ante-

rior, the right, and the left, continue to divide in the same way and at nearly the same time, though the cells of the anterior arm become larger than those of the others, and this entire arm becomes broader, though scarcely as long as either of the others.

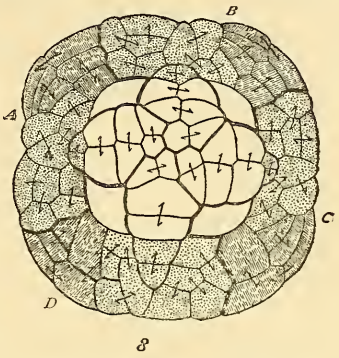


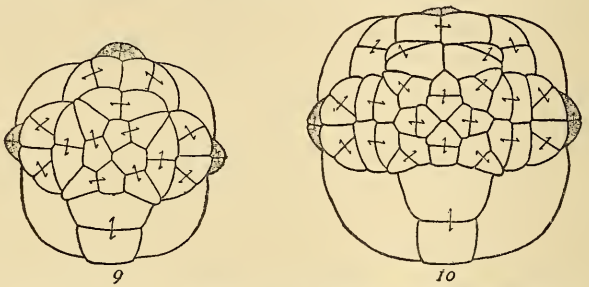
DIAGRAM 8. — Crepidula: 92 ectoblast cells (109-cell stage). Shading and heavy lines as in preceding diagrams. The egg is represented as if all the ectoblast cells could be seen from the apical pole, though actually many of the peripheral cells lie far down on the sides, or even on the ventral face of the egg.

After the stage shown in Diagram 8 the right and left middle cells in each of the three arms just mentioned divide in a purely bilateral manner. These cleavages are not only symmetrical with reference to the median plane of the embryo, but the time at which the cells divide shows that the cleavage is bilateral and not radial, the middle cell on the posterior side of the transverse arms dividing before any of the others, Fig. 49. The right middle cell in each case divides in a dexiotropic direction, the left middle cell in a laeotropic, Figs. 49, 50, and Diagram 9. In this way a *right* and *left intermediate cell*, $1a^{1.2.2.1.1}$ and $1a^{1.2.2.2.1}$, etc., is formed in each of these three arms.

Fig. 50, which is a view from the apical pole, shows the cross after the intermediate cells have been formed. This figure and the next one, Fig. 51, are particularly interesting, since they show a polar body attached at the point where the four apical cells come together. This is the last stage in which I have found the polar bodies attached to the egg, though they are found still later free in the egg capsules and sometimes within the alimentary canal of the embryos.

Very soon after the formation of the intermediate cells the peripheral rosette cells divide almost in a radial direction into central and peripheral portions, $1a^{1.1.2.1}$ and $1a^{1.1.2.2}$, etc., Fig. 51, which are almost equal in size. The division is purely bilateral, and the two posterior cells divide a little before the two anterior ones, as was the case with the middle cells.

After this stage the cleavage at the apical pole becomes more or less irregular, and is especially difficult to follow, because the shape and position of the cells is so variable. In fact, at about the stage shown in Figs. 53–56, the whole egg becomes irregular in outline, and every part seems to be undergoing contraction or expansion. This, like the previous period of irregularity mentioned on p. 75, and shown in Figs. 33, 34, is due to divisions and changes of position which are taking



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DIAGRAMS 9 and 10. — First quartette in Crepidula, showing the later history of the cross and turret cells.

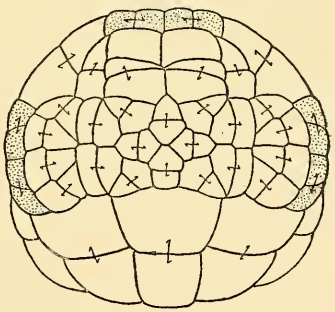
place in the entoblasts. The result of these changes is the formation of the archenteric cavity; and as soon as this is formed, Figs. 63, 64, the egg comes back to a regular form again, and many landmarks which were lost for a time reappear. Some marks, however, especially the cells of the anterior and posterior arms of the cross, can be followed right through this period. Soon after the division of the peripheral rosette cells the apicals divide in a radial direction into two cells about equal in size, Fig. 53 and Diagram 10. These we shall call the *inner* and *outer apicals*, $1a^{1.1.1.1}$ and $1a^{1.1.1.2}$, etc. In this case, as in the two preceding cleavages, the posterior cell of this quartette divides first, the anterior one last.

About the same time that the apicals are dividing, the right and left basals in the transverse arms divide in a direction parallel to the long axis of the arm into the *inner* and *outer basals*, Fig. 53, $1a^{1.1.2.1}$ and $1a^{1.1.2.2}$, etc., and a little later the corresponding cells in the anterior arm divide obliquely and unequally, giving off a thin, wedge-shaped *outer basal*, which lies between

the basal and middle cells of the arm, Figs. 65–68, 70, and Diagram 10. At nearly the same time the anterior intermediate cells divide into upper and lower portions, which lie on each side of the anterior arm, Fig. 56 and Diagram 10.

Next, as shown in Fig. 53, the two cells (proximal and distal tip cells) derived from the tip cell of the posterior arm divide in the direction of the long axis of the arm, forming four cells which have come from the single original tip cell. These four cells lie in line with the two proximal cells of the posterior arm, so that now the entire arm consists of a linear series of six cells.

Finally, the last stage in which I have been able to recognize the entire cross is shown in Fig. 56 and Diagram 11. At this stage the two tip cells which lie at the ends of the right and the left arms divide across the axis of the arms, so that there are four tip cells at the end of each arm. Unlike the four tip cells of the posterior arm, these do not lie in the long axis of the arm, but across it. About the same time all the middle and intermediate cells of the transverse arms divide radially, so that there come to be two right and two left middle cells, and two right and two left intermediate cells in each arm, one of the two being proximal, the other distal in each case.



11

Diagram 11. — The first quartette in Crepidula, showing the later history of the cross and turret cells.

The fate of the tip cells of the anterior arm is very uncertain. I have not been able to trace them satisfactorily beyond the stage shown in Fig. 56, when they are still very small and insignificant. *However, I believe that in C. plana they are crowded entirely out of the layer of ectoblast cells, and that they are thrown wholly away.*[1] Fig. 71, $2b^{1.1.2}$ shows the two anterior tip cells very plainly. Even here, however, they show important changes in position and structure ; they project above the level of the surrounding ectoblast cells, and their nuclei have no definite boundaries, while the chromatin seems to be dissolving and

[1] See Note p. 204.

spreading throughout the cell. The whole cell stains very much more uniformly than does a normal cell, and in this regard these tip cells resemble the polar bodies in the stages where they are last seen and where they are undoubtedly degenerating. Further stages in the degeneration of the tip cells are shown in Figs. 69, 70; in the former the two tip cells are pushed still further above the level of the surrounding cells, while in Fig. 70 they are separated from each other and practically detached from the egg. I have observed this process in only a few eggs of the species C. plana and am not wholly convinced that it is a constant feature. Such a phenomenon is certainly very remarkable and unusual, and I am not prepared to draw any conclusions as to its significance.

In this last stage, then, in which the cross can be recognized as a whole, it is composed of the following cells :

Apical	Inner		4	
	Outer		4	
Peripheral Rosette	Inner		4	
	Outer		4	
				16
Transverse Arms	Basal	Inner	2	
		Outer	2	
	Middle	Inner	2	
		Outer	2	
	Intermediate	Inner	2	
		Outer	2	
	Tip (Terminal)		4	
		Right Arm		16
		Left Arm		16
				32
Anterior Arm	Basal	Inner	2	
		Outer	2	
	Middle		2	
	Intermediate	Inner	2	
		Outer	2	
	Tip (Terminal)		2	
				12
Posterior Arm	Basal		1	
	Middle		1	
	Intermediate		0	
	Tip (Terminal)		4	
				6
		Total		66

I have found it impossible to trace the cell lineage of certain parts of the cross, especially the transverse arms, farther than the stage shown in Figs. 54, 56, and Diagram 11. The cells become so numerous beyond this stage that I can only point out the general outlines of these arms, *e.g.*, their posterior boundary and terminal cells are for a long time clearly marked by the large ciliated cells which lie just behind them, Figs. 53, 55, 64, *et seq.* The anterior borders of the transverse arms become confused with the lateral extensions of the anterior arm, but even in this case it is possible for a considerable time to distinguish between the anterior and transverse arms by means of the large anterior turret cells or their derivatives which lie in the angles between these arms. But while there is a degree of uncertainty about the exact outline of the transverse arms, there are other portions of the cross which remain perfectly distinct until a period much later than any shown in the figures, in fact until the larval life is practically at an end. In all the later figures which show an apical view of the egg, *e.g.*, Figs. 50, 56, 79, the four characteristically arranged apical cells can be plainly seen, while the two proximal cells in the posterior arm and the median portion of the anterior arm can be recognized throughout the greater part of the larval life. The cells of the anterior arm which remain recognizable throughout this period are, counting from the apical cells :

Apical, outer (anterior)		1
Basals	Inner	2
	Outer	2
Median		2
Total		7

The two proximal cells of the posterior arm ($1d^{1.2.1}$ and $1d^{1.2.2}$) are recognizable for a very long period, *e.g.* Fig. 64. They become very large, and, together with the two posterior turret cells, form a large part of the head vesicle or umbrella.

(d) *Significance of the Cross.* — In seeking to learn the significance of this peculiar structure, it will be well first of all to compare it with similar structures found in the segmenting eggs of other animals, then to inquire into the mechanical principles involved in its formation, and finally to seek for its significance in the ontogeny.

Blochmann ('81) has given a most interesting and complete account of the cross in Neritina. He did not recognize that the four apical cells are in any way connected with this structure, and hence he speaks of it as four cell series ("Zellreihen"), an anterior, posterior, right, and left. He followed the history of these cell series until there were three cells in each one except the posterior, which contained four. Owing to the presence of peculiar shining granules in the terminal cells of the transverse arms, Blochmann was able to trace these cells to a very late stage in the cleavage. He believed that they entered into the formation of the velum, and hence called them "Urvelarzellen."

In spite of the many minute and wonderful resemblances between the cross in Neritina and Crepidula, the derivation of the cells composing it is very different in the two animals if Blochmann's account is to be trusted. In Neritina, as in Crepidula, the cross is first recognizable when there are two cells, one basal and one terminal, in each arm. The following scheme shows Blochmann's derivation of the cells of the cross in Neritina, as compared with my account of Crepidula:

NERITINA.	CROSS CELLS.	CREPIDULA.
$1a^{1}$–$1d^{1}$	Apical	$1a^{1.1}$–$1d^{1.1}$
$2a^{1.1}$–$2d^{1.1}$	Basal	$1a^{1.2}$–$1d^{1.2}$
$2a^{1.2.1}$–$2d^{1.2.1}$	Terminal	$2a^{1.1}$–$2d^{1.1}$

As has been mentioned already (p. 65), Blochmann is certainly wrong in the designations given the outer-belt cells, and consequently wrong in the designation of all cells derived from them. Making allowance for this error, we find that the terminal cells are derived from exactly the same source in Neritina and Crepidula. The only other difference concerns the basal cells. In the derivation of these cells Blochmann is certainly in error. Although he expressly states that the basal cells arise from the second quartette, he shows no stages in which the spindles are present, and his figures indicate that the basal cells have arisen exactly as they do in Crepidula and Umbrella, *viz.*, from the apical cells. The position of the cells is the same, and it is highly improbable that their origin is different. As opposed to Blochmann's view, I urge Heymons' careful

observations on Umbrella, and my own results on four species of Crepidula.

Kofoid ('94) also has expressed the view that Blochmann was wrong in the derivation of the basal cells, and as he presents other evidence in favor of the position here taken, I quote his words: "There are indications, however, that they (the basal cells) were really derived from the apical quartette a_1–d_1; for (1) their nuclei are nearer those of the apical quartette; (2) the cells of the apical quartette are much smaller after the cells a''_2–d''_2 appear than before; (3) a'_2–d'_2 have just arisen by a recent division, whereas some time has elapsed since the first division of the apical quartette."

The subsequent division of the basal cells is identical with their division in Crepidula, not only in the direction of the cleavage and the size of the resulting cells, but also in the time of its occurrence. This is the more remarkable when it is considered, as will be done in a moment, that *in both Crepidula and Neritina the direction of this cleavage really determines the continued existence of the cross, and further, that it violates the "law of alternating cleavages."*

In its later history the identity of the cross in Neritina and Crepidula is still further emphasized. At a stage with 49 ectoderm cells (Blochmann's Fig. 56, corresponding approximately to my Fig. 36 or 38), the cells of the posterior arm of the cross divide so as to form a series of four cells, while each of the other arms contains but three. This is such a remarkable agreement with what takes place in Crepidula, that I think it worth while to quote Blochmann's words on this point (p. 158): "Besonders bemerkenswerth erscheint bei diesem Stadium das Auftreten einer vierten Zelle in der aus drei Zellen bestehenden Reihe, die in der Mitte der hinteren Hälfte der Ektodermscheibe verläuft, während in den drei anderen entsprechenden Zellreihen die Dreizahl erhalten bleibt. Das Auftreten dieser Zelle ist ein ganz Konstantes und wurde an fünf Präparaten beobachtet. Man kann wohl sagen, dass in der Ektodermanlage an und für sich erst durch das Auftreten dieser Zelle vorn und hinten unterscheidbar wird, während vorher nur die Richtung der Sagittalachse durch das Vorhandensein der Körnchen-

zellen *vz* und vz_1 in den seitlichen Reihen erkennbar war." Truly such a fact is "especially noteworthy" when it is found reproduced in another very different animal; and, standing as it does in direct relation to the origin of bilateral symmetry, it is a fact of profound significance.

The further history of the cross in Neritina is not given in detail. An ectodermal invagination is described as occurring at the apical pole, and Blochmann's Fig. 59 shows that this invagination includes the four apical cells and all the cells of the transverse arms, except the terminal ones. Judging by the figures the transverse arms seem to have been drawn into this invagination, while the turret cells and the anterior and posterior arms lie outside of it. If such a thing really happens, the transverse arms of the cross must first be wholly separated from all their connections with surrounding cells, and then drawn into the invagination; in fact, the figures mentioned show that this has happened, for the terminal cells of the transverse arms (the "Urvelarzellen") have been drawn inward until they immediately adjoin the turret cells. Concerning this invagination I have already spoken (p. 31), and I need only repeat here that I believe it is not a normal formation.

The two granular tip cells are the only ones which Blochmann was able to trace farther than the stage already mentioned (his Fig. 59). These cells he calls the *Urvelarzellen*, and he states that the velar cells first appear between them, on the dorsal side of the embryo, and then, apparently by the division of the *Urvelarzellen*, they extend ventrally around the anterior end of the embryo. It is almost certain that these same cells form part of the velum in Crepidula (see p. 132).

Heymons ('93) figures the cross plainly enough in Umbrella, as is shown by Diagram 12, *c* and *d*, taken from his Figs. 14 and 20, and yet he does not appear to have recognized this structure. To be sure, he speaks of a cross of ectoblast cells being present, and refers repeatedly to the cross in Neritina and Crepidula, but the cross which he points out in Umbrella is a wholly different thing from that in either of the other forms. It is composed entirely of cells of the second quartette (a″, b″, c″, d″), does not reach the centre of the ectodermal field, and has

wholly different axial relations; besides, the arms are never more than two cells long, though they may become three cells broad. The real cross, *i.e.*, the structure homologous with the cross in Neritina and Crepidula, is plainly present in his figures, and its close resemblance to the same structure described by Blochmann and myself is all the more striking, since apparently Heymons did not recognize its presence.

It is composed of cells of exactly the same derivation and of relatively the same size and position. Thus the terminal cells are, using my nomenclature, $2a^{1.1}$–$2d^{1.1}$, and they arise by laeotropic division; the basal cells are $1a^{1.2}$–$1d^{1.2}$, and they arise by dexiotropic cleavage. The two cells of each arm, and especially their nuclei, lie in line with one of the apical cells, and a line drawn through the nuclei of these three cells forms a curved radius, the four radii being dexiotropic. Heymons especially says of the terminal cells (of course he does not use this designation): "Es sind dies die kleinsten Ektodermzellen welche bisher gebildet wurden." To all of these facts I have already called attention in Crepidula, and in general they seem to be true of Neritina.

In the time of its formation the cross in Umbrella shows some interesting differences from the cross in Neritina and Crepidula, *e.g.*, the terminal cells are first formed and the basal cells are not formed until a considerably later period. The arms are more curved in a dexiotropic direction than in either of the other gasteropods, and the whole cross is less clearly marked off from the surrounding cells. *But most important of all the differences is the fact that the first division of the basal cells is laeotropic in Umbrella, Diagram 12, d, while it is invariably dexiotropic in Crepidula and Neritina.* Upon this difference the future recognizability of the cross in the last-mentioned cases depends. If these basal cells should divide in Neritina and Crepidula, as they do in Umbrella, there would be no cross after the stage in which there are two cells in each arm. The existence of the cross in the later stages depends upon the direction of this one division. It is therefore all the more interesting to note that this division in Umbrella follows the usual rule of alternation of direction, whereas in Neritina and Crepidula it violates that rule.

The cross in Umbrella develops more slowly than in either of the other forms, — thus the basal cells are formed at the 24-cell stage in Neritina, the 25-cell stage in Crepidula, and the 39-cell stage in Umbrella. The first division of the basal cells occurs at the 37-cell stage in Neritina, the 44-cell stage in Crepidula, and the 83-cell stage in Umbrella. On the other hand, the terminal cells are formed when 25 cells are present in Umbrella, 28 in Neritina, and 30 in Crepidula. Likewise the division of the turret cells, which occurs at the 63-cell stage in Umbrella, does not occur until long after the 111-cell stage in Crepidula, at which point I ceased to follow the lineage of the entire egg. But in spite of these two cases in which Umbrella outstrips Crepidula, the division of the cells of the first quartette is much slower in the former than in the latter. Thus there are in Umbrella at the 91-cell stage 16 cells of the first quartette ; in Crepidula, at a corresponding stage, 23 cells. In both cases the greatest activity is in the second quartette. Heymons says of Umbrella : " The micromeres of the first generation are smallest, those of the last largest " (*generation* is used in the sense of quartette). In Crepidula the differences are not marked, though I think the second is somewhat larger, when formed, than either the first or third. The larger size and more rapid division of the cells of any quartette are probably connected with the larger size or more rapid development of the organs to which they give rise, as Lillie ('95) has established in the case of Unio. The velar field (derived from the first quartette) is certainly larger and develops more rapidly in Crepidula than in Umbrella, and corresponding to this we have the larger size of the cells when first formed and their more rapid divisions subsequently. The smaller size of the velar field in Umbrella may account for the relative unimportance of the cross in that animal. Concerning the fate of the cross cells in Umbrella nothing is known.

Heymons has observed, but does not figure, the division of the terminal cells and a second division of the basal cells ; the direction of these divisions is not given. He has also observed the rosette division by which four small cells are formed at the apical pole, " strikingly like the apical rosette of Wilson " ; as

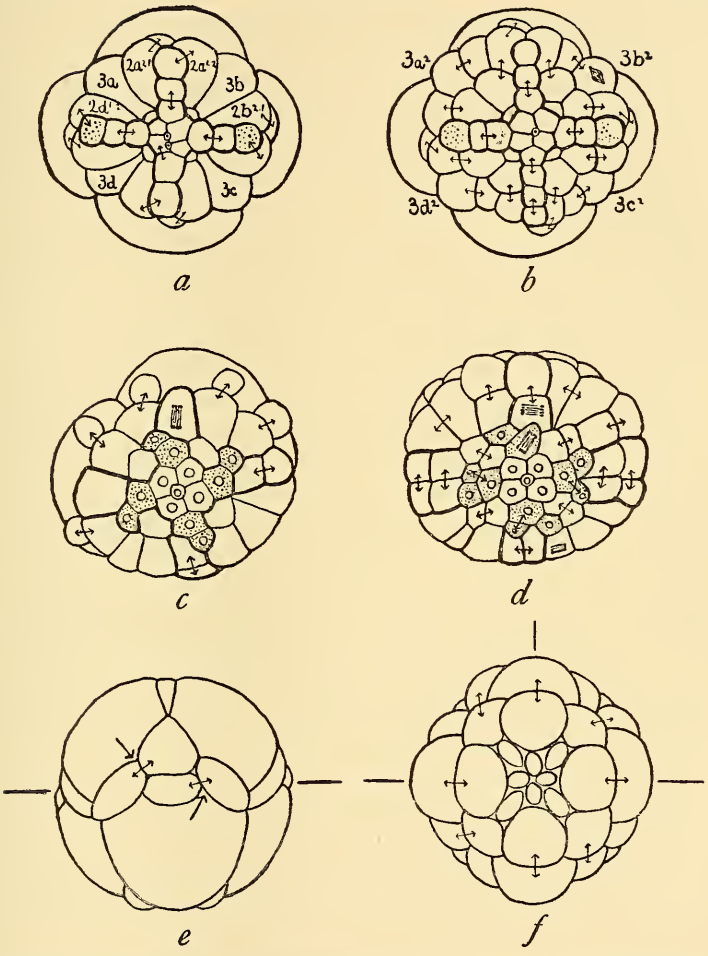


Diagram 12.—The cross in Neritina, Umbrella, and Chiton.—*a*, Neritina: three cells in each arm except the posterior; the granular tip cells of the transverse arms are the "Urvelarzellen." (Blochmann's Fig. 53.)—*b*, Neritina: four cells in the posterior arm, three in each of the others. The probable origin of the outer belt cells is indicated by arrows, and the designation of the cells in this and in the preceding figure are given as in Crepidula. (Blochmann's Fig. 56.)—*c*, Umbrella: the arms of the cross are stippled; Heymons' so-called "cross" is shown in heavy outline. (Heymons' Fig. 14.)—*d*, Umbrella: stippling and outlines as in *c*. The basal cells in the arms of the cross have divided laeotropically, the turret cells bilaterally. (Heymons' Fig. 20.)—*e*, Chiton: lateral view of the 32-cell stage. The small cells around the equator of the egg correspond in origin and position to the *turret* cells and the *tip* cells of the gasteropod; they should form the prototroch if they have the same destiny in the two cases. (Metcalf's Fig. XIV.)—*f*, Chiton: apical view of the 48-cell stage, showing the *cross*, the *rosette*, and the *turret* cells. (Metcalf's Fig. XXIV.)

in Nereis and Crepidula, this is the third division of the apical quartette.

It is very interesting to note that in so primitive a form as Chiton, in which the cleavage in general appears to be very different from the ordinary molluscan type, the cross is present, at least in some species. In Metcalf's ('93) figures of Chiton marmoratus and C. squamosus the cross is found as in the gasteropods just mentioned, being composed of cells of exactly the same cell origin and position, except that the tip cell is shown displaced a little to the right. These facts and several others which will be referred to later are shown in Diagram 12, *e* and *f*, which are copies of Metcalf's Figs. XIV and XXIV.[1]

Wilson ('92) has shown that in the polychaetous annelid Nereis, a cross of ectoblast cells is present which in many respects resembles the cross in Neritina, Umbrella, and Crepidula. This annelidan cross, like the molluscan one, contains two cells in each arm when first formed, and these later increase to three. The centre of the cross, too, is formed by the four apical cells. In its later history each arm of the cross undergoes longitudinal splitting (*cf.* Wilson, Fig. 41), as is the case in Crepidula ; but here the resemblances end. Professor Wilson has shown that the cross in Nereis differs both in origin and destiny from the cross in Neritina, and that both differ from Crepidula. This conclusion as to the difference between the mollusk and the annelid I can only more fully confirm, though, as I have already pointed out, it is almost certain that Blochmann's derivation of the cross in Neritina is wrong, and that it has the same origin, structure, and destiny in Neritina and Crepidula ; the same thing is true of the origin and structure of the cross, at least in the early stages, in Umbrella. But in neither origin, structure, nor destiny does the molluscan cross resemble the annelidan. Wilson seems to consider them alike in structure, for he says (p. 442): "It is certain that,

[1] The cross is beautifully shown in Ishnochiton from the California coast, which is being studied at present by Mr. Harold Heath in the Zoölogical Laboratory of the University of Pennsylvania. This work, which is far the most complete yet done on Chiton, shows that the cleavage in that animal not only belongs to the gasteropod type, but that it is, for a considerable period, cell by cell the same.

although the two crosses have exactly the same structure, they have a completely different origin." From this statement I should be compelled to dissent, for I do not believe that they are alike even in structure. The cross in Nereis is purely radial in position, dexiotropic in the gasteropods. It ultimately lies in the median and transverse planes in the mollusk, midway between these planes in the annelid. It is formed almost entirely from the apical cells in the mollusk, from the terminal cells in Nereis, and corresponding with this difference the terminal cells are much the largest ones in the cross in Nereis, while they are the smallest ones in the gasteropods. And again, the posterior arm in Nereis is like each of the others, whereas in Crepidula and Neritina it becomes very different in structure. The cells composing the cross in Nereis and Crepidula are shown in the following scheme :

Nereis.	Cross Cells.	Crepidula.
$1a^{1.1.1}$ –$1d^{1.1.1}$	Apical	$1a^{1.1}$–$1d^{1.1}$
$1a^{1.1.2.1}$–$1d^{1.1.2.1}$	Basal	$1a^{1.2}$–$1d^{1.2}$
$1a^{1.1.2.2}$–$1d^{1.1.2.2}$	Terminal	$2a^{1.1}$–$2d^{1.1}$

The cross forms relatively much later in Nereis than in Crepidula, as is shown by the exponents used in designating the cells.

In Nereis the arms of the cross are formed entirely from the cells $1a^{1.1.2}$–$1d^{1.1.2}$, which in Crepidula I have called the peripheral rosette. These cells are formed at exactly the same division of the apical quartette in Crepidula and Nereis, *viz.*, the third ; in exactly the same direction, *viz.*, slightly laeotropic, almost radial (Figs. 44 *et seq.*, and Wilson's Fig. 27); and they lie in exactly the same position, *viz.*, between the turret cells (Wilson's trochoblasts) peripherally and the apical cells centrally. In Crepidula the two anterior peripheral rosette cells are secondarily separated from the turret cells by the lateral extension and consequent junction of the arms of the cross ; the two posterior peripheral rosette cells remain in contact with the turret cells, Figs. 49 *et seq.* In both Crepidula and Nereis the peripheral rosette cells, $1a^{1.1.2}$–$1d^{1.1.2}$, divide in nearly the same direction (radial in Nereis; slightly bilateral, almost radial, in Crepidula), forming a cell series in each quadrant which radiates from the apex, Figs. 51, 53, 62, and Diagram 13. These radiat-

ing rows of cells I shall call the *rosette series*, a name suggested by the word *rosette*, first used in this connection by Wilson to designate the small apical cells formed by the third division of the first quartette. The following table will show the cells in Crepidula which correspond to the cross cells of Nereis :

NEREIS.	CREPIDULA.
Rosette	Apicals
Basals	Inner Peripheral Rosette
Middles, Terminals	Outer Peripheral Rosette

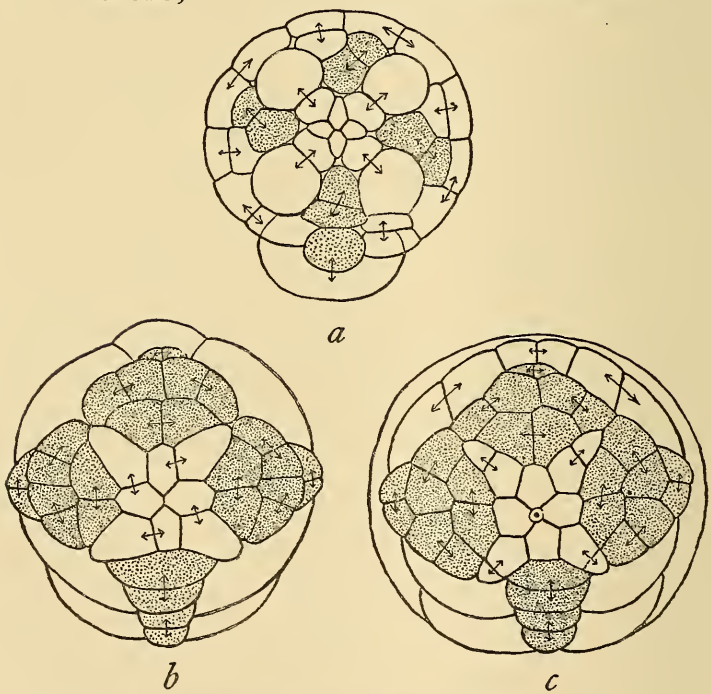


DIAGRAM 13. — The cross in Nereis and Crepidula. — *a*, Nereis : the stippled cells are the *intermediate girdle cells* (molluscan cross) excepting the posterior one (x^3) which corresponds to the "tip cell" in the gasteropod. The trochoblasts lie at the margin of the egg. (Wilson's Diagram 11. B.) — *b*, Crepidula : cross cells (intermediate girdle cells of Nereis) are stippled. Apical and rosette cells unshaded as in *a*. Trochoblasts around margin. — *c*, Crepidula : shading as in *b* ; rosette cells and anterior trochoblasts divided.

Inasmuch as the formation of the peripheral rosette cells occurs late in the cleavage, I have not been able to find the further division of the outer cells of the rosette series in Crepid-

ula which corresponds to the division of the terminals in Nereis, by which the middle cells are formed.

If now we attempt to compare the cells of the molluscan cross with the corresponding cells in Nereis, we find that the basal cells correspond to the *intermediate girdle cells* ($1a^{1.2}$–$1d^{1.2}$) in Nereis, while the terminal cells are represented by cells which lie outside the prototroch, and, except in the case of the posterior arm, are secondarily separated from the intermediate girdle cells by the products of the trochoblasts, which shove in between the terminals and the intermediate girdle cells. About the time that these cells, $2a^{1.1}$–$2d^{1.1}$, are formed in Nereis the intermediate girdle cells divide into an inner and an outer part which correspond to the basal and middle cells in the cross of Crepidula. There are thus formed four radiating rows of cells which run out to the prototroch, and correspond, at least in origin, to the arms of the molluscan cross. In the case of the posterior row there is a plain cell series, with basal, middle, and terminal cells exactly as in Crepidula (*cf.* Diagram 13, *a*, also Wilson's Fig. 38). The terminal cell (x^3) in this case is a product of the first somatoblast and is not separated from the middle cell by the prototroch, as is the case in each of the other cell series. The following table indicates what cells in Nereis correspond to the cross cells in Crepidula:

Crepidula.	Nereis.
Apicals	Rosette
Basals	Intermediate Girdle, Inner
Middles	Intermediate Girdle, Outer
Terminals	Post-trochals and x^3

Every cell of the annelidan cross can be identified with the molluscan, and conversely every cell of the molluscan cross can be identified with that of the annelid. Yet on the other hand, with the exception of the apicals, not a single cell of the annelidan cross is found in the molluscan, nor a single cell of the molluscan cross in that of the annelid. In fact, the arms of the molluscan cross lie midway between the arms of the annelidan cross and *vice versa*. The fact is, there are two distinct systems of radiating cell series at the animal pole in both annelids and mollusks, the one lying in the median and transverse

axes, the other midway between these axes. The former is the well-developed cross of the gasteropods, but is not prominent in annelids (the intermediate girdle cells of Nereis); the latter is the conspicuous cross of the annelids, but the relatively inconspicuous rosette series of the gasteropods. We have to do, therefore, with two totally different crosses — different in position, structure, origin, and destiny. Wilson says (p. 443): "There is every reason to believe that the annelidan and molluscan crosses are analogous, but not homologous structures, whose origin is in some way connected with the mechanical conditions of cleavage. What these conditions are I am unable to conjecture." However, if my interpretation of these structures is correct, the crosses as heretofore defined are not even analogous, and ought not to be compared at all.

In conclusion then, the cross observed by Wilson in Nereis is not to be compared with the molluscan cross, but rather with the rosette series of Crepidula, and conversely the molluscan cross is to be compared with the intermediate girdle cells of Nereis, and not with the cross in that animal. To call things which are not to be compared by the same name, and things which are to be compared by different names, would certainly be confusing, and the word *cross* ought therefore to be changed in either the annelid or the mollusk. Since this word was first used in describing the radiating structure in the molluscan egg, and since it has been found in several genera and species of gasteropods, and is, moreover, such a definite structure that its history can be followed very far through the embryology, I shall retain the word *cross* as heretofore used in the case of molluscan eggs, and shall take the liberty of changing the designation of the so-called "annelidan cross," calling it the *rosette series*.

The cross, as thus defined, is present in the annelid, but is not a prominent structure, as it is in the case of the gasteropods; on the other hand, the rosette series is present in Crepidula as well as in Nereis, but it divides more slowly than in the annelid, and is therefore composed of fewer cells.[1]

[1] In early stages of the cleavage of Ishnochiton, Heath finds that the molluscan cross is typically developed and is very prominent, while in later stages it becomes less marked and the rosette series (annelidan cross) becomes as fully developed and almost as prominent as it is in Nereis.

The annelidan cross (rosette series) has also been described by Mead ('94) in Amphitrite, Lepodonotus, and Clymenella; and in the case of the first two, the division of the "four cells which form the cross is bilateral," as it is in Crepidula, whereas in Nereis it is radial.

We find, therefore, in a large number of annelids and mollusks, representing very widely different orders, that there is a peculiar arrangement of cells at the apical pole which takes the form of two crosses whose cells have in the different animals the same origin, the same axial relations, and (as will be shown later), in many cases at least, the same destiny.

What is the significance of these crosses? Are they the necessary result of alternating cleavages, surface tension, and the like, or must we seek their cause in more remote and obscure phenomena? As was just mentioned, Wilson finds that their "origin is in some way connected with the mechanical conditions of cleavage." This conclusion is unquestionable, but that their characteristic and peculiar features are entirely *caused* by such conditions, as such a statement might seem to imply, is very questionable indeed.

In the first place it may be well to call attention to the fact that these radiating structures are not caused by the crowding of cells into the furrows between the macromeres. This is well shown in Neritina, Paludina, Crepidula, and Nereis, in which the cross is first formed midway between those furrows. In Umbrella, indeed, it is first seen lying over the furrows between the macromeres, — a position which it ultimately takes in Crepidula, owing to the rotation of the entire cap of ectoblast, — but there is no probability that even in Umbrella the cross is caused by those furrows.

The cross and the rosette series are the direct result of the *position*, *size*, and *shape* of their constituent cells. Anything that will satisfactorily explain these three things will afford a satisfactory answer as to the significance of these structures.

The position of cells in general is due to the direction of the cleavages, and to the subsequent rotations of the division products. The shape of cells also may be explained in general as the result of their size and position. The size of cells, however,

is not so easily explained. No phenomenon is more common than the unequal division of apparently homogeneous cells, but none is more difficult of explanation on the grounds of a purely mechanical theory of development. All these general phenomena might be perhaps the result of known mechanical conditions; and yet the combinations, modifications, and coördinations of these processes, which appear in the formation of almost any structure, ultimately require some explanation other than mechanics can as yet supply. This is abundantly illustrated in the whole history of the cleavage of such an egg as that which we are considering, and nowhere better than in the formation of the cross.

Up to the time when there are two cells in each arm of the cross, the position of each cell may be attributed, at least in part, to the regular alternation in direction of successive cleavages. The next step in the formation of the cross is highly peculiar; the basal cells, which were formed by dexiotropic cleavage, divide in a dexiotropic direction in both Neritina and Crepidula. In Umbrella, on the other hand, this division is laeotropic, as it should be, according to the rule that successive cleavages are in opposite directions. Associated with this regular alternation of cleavage in Umbrella is the fact that immediately before the division of the basal cells, the turrets divide almost bilaterally; whereas they remain undivided in Neritina and Crepidula, in which the cleavage of the basals is reversed. I was therefore inclined, at first, to attribute this reversal to the lateral pressure of the undivided turrets upon the basals, but several considerations have convinced me that this cannot be the case; in the first place the basals show no signs of such pressure, being full, well-rounded cells; again the turret cells in Neritina are not large enough to exert any considerable lateral pressure upon the arms of the cross; and, finally, even if such pressure were exerted, it would deflect the spindles only a few degrees from the normal position, and would still leave them laeotropic, whereas they are distinctly dexiotropic.

The same considerations are applicable to the history of the posterior arm, where repeated divisions are always in the same direction, as is true of teloblastic growth in general, and also to many of the later cleavages where reversals occur again and

again, especially with the appearance of bilateral symmetry. In all such cases, the direction of cleavage and the consequent position of cells is due to something other than the alternation of cleavage, surface tension, or intercellular pressure. For the present, therefore, one is justified in assuming that these peculiarities in the direction of cleavage, and in the *position* of resulting cells, is the result of intrinsic rather than of extrinsic causes.

This conclusion holds true with especial force in the study of the relative *sizes* of the cells composing the cross, and other adjacent structures. The very small size of the tip cells has been emphasized by Heymons and myself ; upon their size depend in part the shape and structure of the entire cross. The more rapid divisions and consequent smaller size of the cells of the anterior, the right, and the left arms, as compared with those of the posterior arm, the great size of the turret cells and of the anterior cell plate, — all these contribute to the most characteristic features of the cross ; and yet the known mechanical conditions of cleavage are wholly unable to explain them.

On the other hand, it is certain that the size of many of these blastomeres can be directly correlated with their prospective functions (*e.g.*, the cells of the posterior arm, the turret cells, the apical cells, the anterior cell plate), and while it is not possible at present to explain all the characters of the cross and the rosette series in this way, a strong presumption is created that these structures, like teloblastic rows of cells, are to be explained as a precocious development of certain parts. The cells of the right and left arms and their derivatives have apparently the same fate, while the destiny of the cells of the anterior and posterior arms differs from that of the transverse arms and from each other. The most obvious significance of the cross is that its cells represent the protoblasts of certain structures which are ultimately to lie in the median and transverse axes of the larva. The identity of the right and left arms is correlated with the fact that the organs are identical on the right and left sides. For a long time the anterior arm is identical with the right and left arms ; in its later stages, however, it becomes slightly different, and in the end gives rise to somewhat different organs. From an early stage the posterior arm

differs from the other three, and correspondingly we find it gives rise to parts of the embryo wholly unlike those which arise from either of the other arms.

The significance of the cross, therefore, as indeed of all the most important features of the cleavage, is prospective; its cause is to be sought in some peculiarity of protoplasmic structure rather than in any extrinsic mechanical factors.

2. *The Turret Cells* (*Trochoblasts*).

The turret cells were formed by the first division of the first quartette of micromeres. Until the tip cells of the cross are formed they are much the smallest cells in the entire egg. Gradually, however, they increase in size, until they become much the largest cells of the egg, excepting the yolk cells. This remarkable increase in size is not due to the fact that they grow so much more rapidly than other cells, for this they do not do, but to the fact that their growth is continuous, and not interrupted by any divisions until a very late stage.

The turret cells lie in the angles between the arms of the cross. Until a late stage they are in contact with the apical cells from which they sprung; but with the longitudinal splitting of the arms of the cross and the formation of the rosette series they are pushed away from the apical cells, though they continue to lie in the angles between the arms. The two posterior turrets hold this position as long as they can be recognized at all. The anterior ones are crowded farther and farther outward and downward by the cells derived from the anterior and transverse arms.

I have never seen the turret cells in process of division, but believe that the anterior ones divide at about the stage shown in Figs. 49, 50, Diagrams 9 and 10; in the earlier figures the division has not taken place, in the later ones it has. The posterior turret cells divide very seldom, if at all. They remain very large, much larger than the anterior ones, and lie on each side in the angle between the anterior and posterior branches of the velum; they ultimately assist in forming the walls of the head vesicle. Certain large cells adjoining the posterior turrets appear to have come from the latter by division, but I do not know that this is true.

These cells, which are altogether characteristic in appearance among the gasteropods, have been found in Neritina, Umbrella, four species of Crepidula, Urosalpinx, and Fulgur. In all these cases they are particularly notable because of their small size. Cells of the same origin and position are found in Chiton, Unio, Nereis, Amphitrite, Lepidonotus, and Clymenella. In all the annelids mentioned it has been found that they form either the whole or a part of the prototroch. In four species of Crepidula, at least the two anterior ones form a portion of the preoral velum, and this is probably true of the two posterior ones also. In no other mollusk has their destiny been determined, but it is highly probable that it is the same in all the gasteropods mentioned, since these cells are wonderfully alike in origin, position, size, and general appearance in all these cases. *Considered in the light of their origin, history, and destiny, it is almost certain that the turret cells of the gasteropods are homologous with the trochoblasts of the annelids.*

In all the annelids named these cells divide twice, and then, according to Mead ('94), in Amphitrite, Lepidonotus, and Clymenella, "stop dividing forever." In Umbrella, Unio, and Chiton they have been seen to divide once only.[1] In Crepidula I have never seen them divide, though I believe the anterior ones do divide at a late stage (Fig. 50). In the other forms their divisions have never been seen.

This is certainly a very remarkable history. Here are four cells which divide at most two or three times, and then probably never divide again, while adjoining cells divide many times and continue this process for a long period. In Nereis while these trochoblasts are producing sixteen cells, the apical cells produce twenty-eight ; in Crepidula, during the time that they are producing six or at most eight cells, the apical cells give rise to forty-two.

These cells are smaller when formed, and divide much more slowly in the gasteropods than among the annelids. This, I believe, is due to the fact that the velum is established relatively much later among the gasteropods than is the prototroch among the annelids.

[1] In Ishnochiton Heath has observed that they divide several times, before entering into the formation of the velum.

The repeated division of small cells like the apical and tip cells, when others like the turrets, ten or twenty times as large, remain undivided, suggests an inquiry into the cause or stimulus of cleavage in a normal egg. The difference between the turret and apical cells, for example, is not to be found in the fact that one is laden with yolk or food material, while the other is not. Both are protoplasmic cells derived from the first quartette of ectomeres, lying on the same side of the egg, for a long time in close contact, with apparently the same conditions of nutrition, growth, and external environment, the differences of size in the early stages being the reverse of those in the later; and yet the smaller cell grows continually and does not divide at all, and the larger cell, while growing no more than the other, divides repeatedly, producing, at the stage shown in Diagram 15, twelve cells, whose total mass scarcely exceeds that of a single posterior turret. What the normal stimulus to cleavage may be is not definitely known, but to any one who will attentively study any definite and regular cleavage it will be abundantly evident, I think, that the stimulus is not to be found in external environment alone, but rather in internal conditions. How any one can follow the history of the blastomeres of an ovum like that of Crepidula, and still maintain that the peculiarities of each cell are due entirely to external conditions or to intercellular relations, is more than I can understand. To me it seems absolutely necessary to believe that *between cells with such different histories there must be some internal or constitutional difference.*

The cause of the small number of divisions of these cells and of their large size in both annelids and mollusks is correlated with their prospective destiny. And, at least among the mollusks, I believe that a law might be formulated to the effect that *the size of cells in general, the frequency and direction of their divisions, and the size of the resulting cell products are all correlated with the ultimate uses to which these cells are put.*[1]

[1] Lillie ('95) has advanced a similar view in the case of Unio, and supports it by a number of observations in which he shows conclusively that there is a close relation between the size of a blastomere and the size of the part to which it gives rise. The ground here taken is merely an extension of Lillie's proposition. It is not always true that the size of a blastomere when first formed is proportional

3. *Organs formed from the First Quartette.*

The following organs, which I have studied with more or less care, are formed from the first quartette : the umbrella, or "head vesicle," an apical plate of ciliated cells, the posterior cell plate, a portion of the velum, the supraoesophageal ganglia, an apical sense organ, a commissure connecting the ganglia with each other and with the apical organ, the cerebro-pedal connectives, and the eyes.

(a) *The Head Vesicle* reaches its maximum development before the veligers escape from the egg capsules; in fact it decreases in size as the velum increases, the walls of the vesicle being drawn out into the velar lobes. In its fully formed condition it is a large bladder-like structure, filled with a transparent fluid. The walls of the vesicle are but one cell thick in early stages, though in later stages a few scattering cells, probably mesoderm, are found on its inner surface. As the head vesicle is formed the apical cells are pushed farther and farther forward, and the vesicle is composed almost entirely of the large ciliated cells which lie posterior to the transverse arms, *viz.*, the posterior turrets and the basal and middle cells of the posterior arm. These cells form a more or less definite structure, lying posterior to the apex, which I have designated the posterior cell plate (P-C., Figs. 74–82).

(b) *The Apical Sense Organ.* — The four apical cells can be still recognized in Fig. 79. In this figure, and also Figs. 78 and 96, it can be seen that these cells are somewhat indented over their outer surface, and have proliferated a few cells inward into the cavity of the head vesicle. This mass of cells, together with the four apical cells from which it arose, forms an organ which soon comes into relation with the supraoesophageal ganglia by means of a strand of cells which grows out from those ganglia. This structure is, I believe, an apical sense organ, and it is located exactly at the point at which the polar

to the size of the part to which it gives rise, as is shown by the case of the trochoblasts cited above, but it is frequently true that the initial size of a blastomere is directly related to the size of the part to which it gives rise *and to the time of its formation.*

bodies were extruded. The apical cells, like many of the surrounding cells, are covered by a coat of fine cilia, but there is no bunch of very large cilia at this point, as in many of the trochophore larvae. An apical sense organ has not hitherto been found in molluscan larvae, I believe.

(c) *The Cerebral Ganglia and Eyes.* — These ganglia are formed on each side of the upper hemisphere, just apical to the row of velar cells and about midway between the anterior and transverse arm, Figs. 78 and 79. Before they begin to form, the cells in this region become quite small by repeated divisions. The method in which the ganglia are formed is shown in section in Figs. 94 and 96, where it is seen that the cells proliferate inward from the surface, and thus form a solid aggregate of cells. Over the area where the ganglia are being formed the ectoderm is slightly depressed, but there is no invagination.

From the position of these ganglia on each side of the anterior cell plate, and in front of the cells derived from the transverse arms of the cross (Fig. 79), it is very probable that they arise from the two anterior rosette series and perhaps in part from the lateral extensions of the anterior arm. In the larva they lie on the ventral side of the coronal plane, and it is therefore probable that they are formed from cells lying originally on the anterior side of the apex.

There are scarcely any data for determining the cell origin of these ganglia in other animals. Von Wistinghausen ('91) states that they are the only derivatives of the first quartette of ectomeres, but Wilson ('92) has shown that this is altogether improbable. In Nereis, Wilson derives these ganglia from a broad cell plate (see his Fig. 86 and Diagram 5) running across the apical pole in a coronal direction and extending as far down on each side as the prototroch. The position of this plate is strikingly like that of the ganglia, commissures, and apical organ in Crepidula as shown in apical view, Fig. 79 ; there is scarcely a doubt that these three organs in Crepidula are homologous with the "cephalic neural plate" in Nereis.

The eyes are formed in connection with the cerebral ganglia as independent involutions of the ectoblast. They lie, as shown in Fig. 104, on the outer side of the cerebral ganglia

and some distance below the surface. The cells of the optic cup which lie farthest to the right and left are the clear lens cells. The cells at the bottom of the cup contain a black pigment which is laid down at their inner ends.

(d) *The Cerebral Commissure. Figs. 76, 79–82, 96.*—The commissure between the two ganglia is formed by an outgrowth of elongated cells from the ganglia themselves. These outgrowths meet at the apical organ, forming a V-shaped structure, the apical organ lying at the apex of the V. Later the two limbs of the V fuse farther and farther away from the organ, forming a Y, and finally a T. The bar of the T is the cerebral commissure, and its stem represents the fused processes which run from the middle of the commissure to the apical organ. The fused character of this process is clearly seen in all the later stages, where its double nature is plainly visible; each half is composed of only a single row of elongated fusiform cells. Still later, with the degeneration of the apical organ, the stem of the T disappears completely, leaving only the commissure.

Similar strands of cells are found in other trochophore larvae, *e.g.*, in Teredo ('80) and Eupomatus ('86), according to Hatschek, but they are said to be muscles, and not nerves. The fact that in Crepidula these strands of cells arise from the cerebral ganglia, and form, in part, the cerebral commissure, is sufficient to prove that they are not muscles. I have, besides, carefully studied the living embryos with regard to this point, and have never seen any evidence of contraction in these cell strands. I am therefore convinced that they are nervous structures, and am consequently inclined to assign to the apical organ, to which these cell strands run, a sensory function.

(e) *The Cerebro-pedal Connectives. Figs. 76, 80–82, 97, 104.* — A process of cells, similar to that which forms the cerebral commissure, grows out from the ventral side of each cerebral ganglion, and extends on each side of the oesophagus into the foot, where it comes into close contact with the ectoderm at the sides of the foot. This is the cerebro-pedal connective. It is formed before there are any pedal ganglia, and it is possible that those structures arise from cells which have come down from the cerebral ganglia with the connective cells.

Even in the oldest stages which I have drawn, Fig. 104, there is no indication of a pedal ganglion, except a few cells which lie between the ectoderm and the otocysts, and which have evidently come from the connectives.

(f) *The Apical Cell Plate.* — The origin of the apical plate from seven cells of the anterior arm of the cross has already been described (p. 91). These cells become covered with a coat of fine cilia, and form a very definite plate, extending from the apical organ to the velum. They are especially notable in that they remain very large, and do not divide during a period when all the surrounding cells are dividing rapidly, and are relatively quite small. In later stages, Figs. 65 and 67, the anterior apical cell is crowded forward between the basal cells of the anterior arm, and in still later stages, Fig. 79, the inner basal cells divide, and two other large cells (probably derived from the outer apicals) are found on each side of the four apicals. I have followed this plate of cells through to the free veliger stage, but have not determined its ultimate destination in the post-larval period.

A plate of such definite and peculiar structure must have, I think, some special significance, and I believe it deserves to rank as a larval organ, though I do not know what function it subserves. I have called it the "apical plate" because of its position and structure, and have not intended thereby to assert its homology with the "Scheitelplatte" of the annelid trochophore. Its resemblance to the "Scheitelplatte" is suggested by its position, by its being covered with cilia, and by its relation to the apical thickening, which forms the apical organ. It is unlike the "Scheitelplatte," as described by Hatschek ('78), in that it lies chiefly in front of the apical pole, and does not form the supraoesophageal ganglia. On the other hand, as has been pointed out (p. 110), there is no doubt that the "cephalic neural plate" of Nereis corresponds in position, in destiny, and probably in cell origin, to the cerebral ganglia, commissures, and apical organ in Crepidula.

(g) *The Preoral Velum (Prototroch).* — The velum is first plainly recognizable at a comparatively late stage, Figs. 65 *et seq.*, and at a time when there are several hundred cells present.

Consequently I have found it impossible to trace with certainty its entire cell origin. Nevertheless the derivation of some of the velar cells can be established with great probability because of their relation to the arms of the cross. I shall describe here merely the preoral portion of the velum, or the prototroch, which is derived in part from the first quartette.

The first velar row, or prototroch, is derived on the anterior side from the cells immediately surrounding the cross. These cells are: (1) the anterior turrets between the arms of the cross, and (2) some of the second quartette cells at the ends of the arms. These cells are shown in position in Fig. 50, forming a single row of cells surrounding the cross on its anterior side. The turret cells, which, as we have seen, correspond in origin to the trochoblasts of the annelids, form the portions of the prototroch between the arms, while the portions at the ends of the arms are derived from the second quartette (see p. 132).

In Figs. 77, 78, and all the later stages, it can be plainly seen that the velum is divided on the dorsal side of the embryo into a posterior branch, P-B, and an anterior one, A-B. The former runs around the edge of the umbrella, and surrounds all of the first-quartette cells; the latter runs up on each side from the edge of the umbrella nearly to the apical organ. This anterior branch, therefore, is composed of cells derived from the first quartette. The position of the cells which form this branch of the velum, relative to the large ciliated cells of the posterior cell plate, P-C, Figs. 77, 78, *et seq.*, shows that they are derived chiefly, if not entirely, from cells of the transverse arms of the cross. As the velum belongs largely to the second quartette, we shall consider its origin, structure, and relationships more fully in the section devoted to those cells (p. 132).

V. History of the Second and Third Quartettes of Ectomeres.

In Crepidula there are no prominent landmarks among the cells of these quartettes, as there are in Nereis, Umbrella, and Unio, and on this account it is difficult to follow the lineage of these cells very far. I have been compelled to use the arms

of the cross as such landmarks ; and as long as any portion of them can be seen the cells of these quartettes can be identified, but when the ectoblast has grown around the egg so that the arms of the cross are no longer visible from the ventral side, I have found it impossible to identify individual cells. Consequently I have not traced many of the cells of this quartette directly to the organs which they form, though I have followed the lineage until there are eleven cells of the second quartette in each quadrant and six of the third, or sixty-eight cells in all. A few cells of the second quartette could be traced farther than this, owing to their relation to the anterior arm of the cross.

The derivatives of these two quartettes form all of the ectodermal covering of the body posterior to the prototroch, and in addition they give rise to the ectodermal portions of the following specific structures : a large part of the velum ; the blastopore, stomodaeum, and mouth ; a region of apical growth at the posterior end of the embryo, the anal cells and proctodaeum, the external excretory cells, the shell gland, foot, and otocysts, the branchial chamber, gills, and larval heart. It will thus be seen that a large part of the important organs, both of the larva and of the adult, are derived from these two quartettes.

The prototroch forms a convenient and fairly accurate boundary between the cells of the first quartette on the one side and those of the second and third quartettes on the other. A glance at Figs. 78 and 80 will show that the portion of the larva posterior to the velum is much larger than that anterior to it, and at the time when the larva changes into the adult the portion of the body anterior to the velum becomes very small and almost disappears, while the region posterior to the velum gives rise to practically the entire body.

1. *The Second Quartette.*

In the formation of the adult body this group of cells is perhaps the most important of any in the entire egg. Knowing this fact, I have done my best to trace the lineage of these cells as far as possible, but in spite of prolonged effort I have not been able to carry the lineage beyond a stage in which

forty-four cells of this quartette are present ; this has been due both to the great number of cells in the entire egg and to the lack of landmarks to which I have already referred.

The first division of the cells of the second quartette has been described (p. 63) ; by it each cell is equally divided in a dexiotropic direction into right and left halves ($2a^1$ and $2a^2$, etc.), Fig. 18 and Diagram 4.

At the second division, which was described on p. 83, the right half is unequally divided, in a laeotropic direction, into a small upper and a large lower cell ($2a^{1.1}$ and $2a^{1.2}$, etc.), Figs. 26–28 and Diagram 6. The upper cell in each quadrant forms the terminal cell in one of the arms of the cross. At the same time, Figs. 26–28 and Diagram 6, the left half ($2a^2$, etc.) divides into an upper and a lower cell ($2a^{2.1}$ and $2a^{2.2}$, etc.) by a cleavage which is slightly laeotropic, almost radial. Of these two cells the upper one is slightly the larger. There are now four cells of this quartette in each quadrant, a right upper and lower (the right upper is the tip cell) and a left upper and lower, Diagram 10 and Figs. 29–33.

Next the right lower and left upper ($2a^{1.2}$ and $2a^{2.1}$, etc.) divide simultaneously in each quadrant, though in the posterior quadrant the division is later than in the other three, Figs. 35, 38, 39, and Diagram 7. In each case the direction of the cleavage is slightly laeotropic in the right cell and dexiotropic in the left. The previous division of the right half (to form the tip cells) was laeotropic, so that here we have another violation of the law of alternating cleavages. By this division six cells in each quadrant are formed,—an upper, middle, and lower right, and an upper, middle, and lower left, Diagram 7 and Figs. 40, 43.

The first of these six cells to divide is the upper right or the tip cells in the arms of the cross. The tip cell of the posterior arm divides before the others, Fig. 42, in a slightly dexiotropic direction. The other tip cells divide a little later, Figs. 44, 45, in a direction which is more or less variable, being usually, however, dexiotropic in the left and anterior arms and laeotropic in the right. Inasmuch as all these cells were formed by laeotropic cleavage, the subsequent division of one of them

in the same direction is a violation of the rule of alternating cleavages; but to just the extent that this cleavage ceases to be perfectly spiral, it becomes bilateral. This division of the tip cells is an equal one, but the posterior tip cells are much larger than any of the others. At a later stage, Fig. 53, the two tip cells of the posterior arm again divide, and this time also in the same direction as the preceding cleavage. While the law of alternation is thereby violated, bilateral and teloblastic cleavages are established.

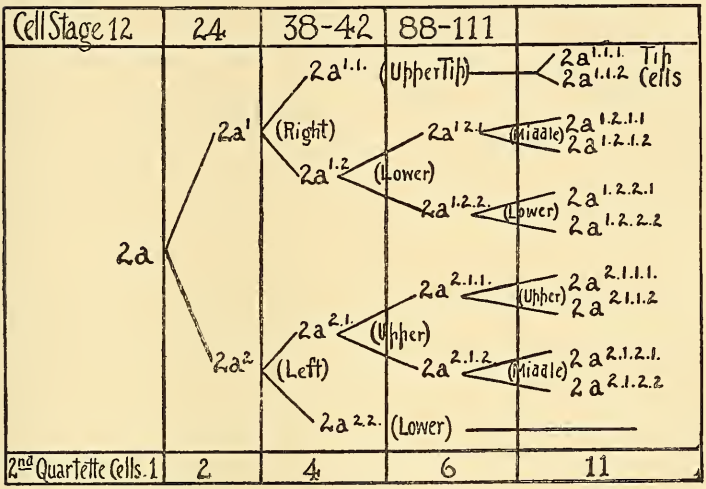
Very soon after this division of the tip cells, the lower right cell ($2a^{1.2.2}$, etc.) in each quadrant divides in a laeotropic direction into two cells ($2a^{1.2.2.1}$ and $2a^{1.2.2.2}$), of which the upper is somewhat the larger; and immediately following this division the right middle cell ($2a^{1.2.1}$, etc.) divides in the same direction into two cells ($2a^{1.2.1.1}$ and $2a^{1.2.1.2}$, etc.), of which the upper one is slightly the smaller, Figs. 46, 47. Coincident with this last division the left upper and middle cells in each quadrant ($2a^{2.1.1}$ and $2a^{2.1.2}$, etc.) divide in a horizontal direction into approximately equal products, Figs. 46, 47, and Diagram 8.

Of the six cells described on the previous page and shown in Diagram 7, all have now subdivided except the lower left one in each quadrant. There are thus formed eleven cells of the second quartette in each quadrant, or forty-four cells in all.

The divisions of this quartette have been in pairs both as to time and direction of cleavage. The table on the following page summarizes the history of this quartette.

Beyond this stage I have not traced the lineage of the entire quartette. I believe, however, that the progeny of some of these cells can be recognized at a more advanced stage, though I have not seen the spindles by which they are formed. In Fig. 56, for example, each of the two terminal cells in the right and left arms of the cross ($2a^{1.1.1.2}$ and $2c^{1.1.1.2}$, etc.) has divided in a bilateral way, forming a row of four cells running around the end of each arm. The terminal cells of the anterior arm are very small and apparently do not divide ; their peculiar history in C. plana has been described (p. 89). In Fig. 50 the cells lying just anterior to the tip cells are probably $2b^{1.2.2.1.1}$ and $2b^{1.2.2.1.2}$, and, accordingly, the large cell lying peripherally to

these ($2b^{2.2}$, Fig. 56) should be the right lower cell shown in Fig. 47. Still the whole identification of the cells of this region must be considered as more or less doubtful. It is, however,



much more certain that the large cells $2b^{1.2.2.1.1}$ and $2b^{1.2.2.1.2}$ of Figs. 62, 63, 69, 70, 71 are the same cells in each of these cases, and that they are the protoblasts of the velum in this region of the embryo.[1]

Comparisons.

Blochmann ('81) has described the divisions of the cells of this quartette in Neritina up to the stage when there are four cells in each of the quadrants, but while his figures agree perfectly with Heymons' ('93) work on Umbrella and my own on Crepidula, his interpretation of the figures is at variance with the results of all recent studies on the cell lineage of gasteropods. Reason has already been given (p. 65) for believing that Blochmann was mistaken in his derivation of some of these cells; and a correction of his interpretation has been suggested which, while finding confirmation in his figures, would bring his account into agreement with the work, particularly, of Heymons, Kofoid, and myself.

[1] See Note p. 204.

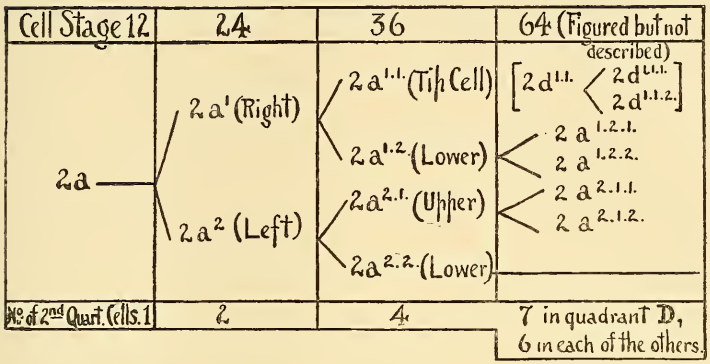
Although no description is given of the further history of these cells in Neritina, Blochmann figures a much more advanced stage (his Fig. 56), in which only the macromeres and the "Urvelarzellen" are labelled. Although he has not described the derivation of any of the twelve new micromeres in this figure, so faithfully is it drawn, that it is possible by comparing it with similar stages in Crepidula to determine the origin of each one of these new micromeres. I have reproduced this figure in Diagram 12, *b*, and inasmuch as its cells are not labelled in the original I have simply designated the cells as in Crepidula, and have indicated their origin by arrows.

It will be seen that two new cells in each quadrant lie just outside the turret cells and between adjacent arms of the cross. These cells correspond, I believe, to the ones which in Crepidula I have called the *middle right* ($2a^{1.2.1}$, etc.) and the *upper left* ($2a^{2.1.1}$, etc.). If this interpretation is correct, there are at the most advanced stage in which the cells can be identified six cells of the second quartette in each quadrant of Neritina. These cells correspond in every respect to the six cells of this quartette which are found in each quadrant in Crepidula, *viz.*, the *lower*, *middle*, and *upper right*, and the *lower*, *middle*, and *upper left*.

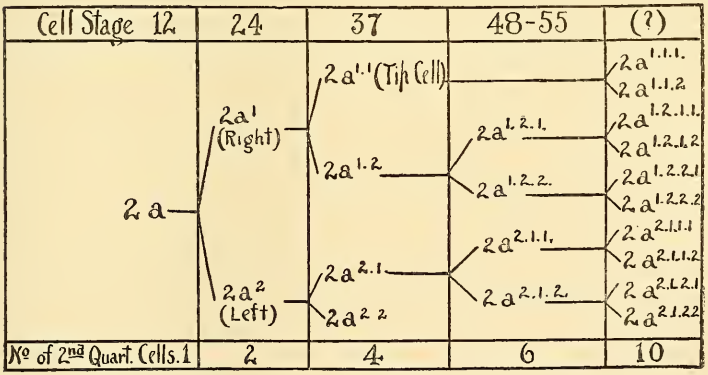
The following table shows the divisions of this quartette as given by Blochmann, and the corresponding cells in Crepidula enclosed in brackets :

Cell Stage 12	24	28	36 – 1
$a^2(2a)$	$a^I_2(2a^1)$	$a^I_2(2a^1)$	$a^I_2(2a^{1.2.})$
			a^{III}_2 (2a$^{1.1.}$ Tip Cell)
	$a^2(3a)$	$a^{II}_2(1a^{1.2})$	$a^2(3a^1)$
			$a^{IV}_2(3a^2)$
№ of 2nd Quart. Cells. 1	2	3	5

Adopting the amendments which have been suggested, the divisions of 2a in Neritina are as follows :



Heymons ('93) has observed every division of this quartette in Umbrella up to the formation of ten cells in each quadrant (he merely mentions the last two divisions, and does not figure or describe them). *These divisions are cell for cell exactly like those in Crepidula. Even the direction of every cleavage and the size of all the resulting cells are the same.* This wonderful and long-preserved resemblance is more particularly shown in the table of cleavages in Umbrella.

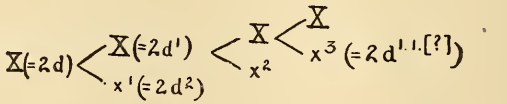


With the exception of one division of the upper left cell in each quadrant, Heymons has seen every division of this quartette which I have observed in Crepidula. Inasmuch as he merely mentions the fact that he has observed divisions of the

cells $2a^{1.1}$, etc. (the tip cells), and does not show them in his figures, I cannot determine whether the posterior arm in Umbrella differs from the other arms, as it does in Crepidula. In conclusion, the wonderful resemblance between Umbrella and Crepidula in the history of the first quartette is shown to a still greater extent in the history of the second.

In both Nereis and Unio the cells of this quartette in quadrant D are unlike those of the gasteropods described. The cell 2d is called by Wilson ('92) the "first somatoblast"; he has followed it through a great many divisions, and has established the fact that it gives rise to a large part of the ectoderm of the trunk. This cell has a remarkably similar history in Unio (Lillie, '95). It divides repeatedly, always in a bilateral way, and apparently gives rise to parts of the body corresponding to those which come from this cell in Nereis.

In our present state of knowledge it is useless to attempt to compare the bilateral cleavages of the first somatoblast in Nereis and Unio with the spiral cleavages of the cell 2d in Neritina, Umbrella, and Crepidula. A few of the earlier division products may perhaps be compared; *e.g.*, in both Nereis and Unio the first three cleavages give rise to similar cells, and at least two of these cells, possibly three, may be compared with the products of 2d in the gasteropods, as is indicated in the following table:



From its peculiar position both with regard to the somatoblast and the cells which correspond to the molluscan cross, I believe that x^3 is the equivalent of the posterior tip cell in the gasteropods.

The division of the other members of the second quartette, *i.e.*, 2a, 2b, and 2c, can be compared with the divisions in the gasteropods much more satisfactorily. In Nereis the divisions of these cells are shown by the following table giving the lineage of 2a:

Cell Stage 16	32	58
2a	2a¹	2a¹·¹·, 2a¹·²· } Post Trochal cells.
	2a²	Left Stomatoblast.
No of 2nd Quart Cells. 1	2	3

The cell $2a^{1.1}$ is the tip cell in Crepidula, and lies in the row of velar cells, not posterior to it. This is not however a profound difference, as will be shown in the section devoted to the velum, since at most it means that at certain points the velum in Crepidula lies *one cell* farther from the apical pole than in Nereis. The cell $2a^2$, with the corresponding cells $2b^2$ and $2c^2$, forms the stomodaeum in Nereis. In Crepidula a very long time intervenes between the origin of the cell $2a^2$ and the formation of the stomodaeum, and I cannot trace the derivatives of this cell into that structure.

In general it may be said that the divisions of the cells 2a, 2b, and 2c, as far as they have been followed, are very much the same in the annelid and gasteropod.

In Unio Lillie ('93) has found that the cell $2a^1$ has a peculiar history: the right half, $2a^2$, takes part in the formation of the larval mantle; the left half, $2a^1$, which in Nereis is the left stomatoblast, moves into the cleavage cavity and there divides, giving rise to the mesoblast which enters into the larval organs. In Crepidula a similar larval mesoblast cell arises in each of the quadrants A, B, and C, and probably from the cell groups derived from 2a, 2b, and 2c (see p. 149). Because of its peculiar history the divisions of the cell $2a^1$ in Unio are unlike those of the corresponding cells in the other quadrants and also unlike the divisions of 2a in any other animal hitherto described. The divisions, however, of 2b and 2c are like those in Neritina and the other gasteropods already described, as the following table of the lineage of 2b in Unio will show:

Cell Stage 12	22	38
2b	2b¹	2b¹·¹·, 2b¹·²· } Larval Mantle.
	2b²	2·b 2.1., 2·b 2.2 } Larval Mantle.
1	2	4

In summing up the history of the second quartette attention should be called to the fact that in the gasteropods, which have been studied with reference to the cell lineage, there is no marked difference in size between the members of this quartette, and accordingly the divisions up to the time when there are eleven cells in each quadrant are almost identically the same in each of the four groups. On the other hand, in the annelids generally and in Unio, at least, among the mollusks, the posterior member, 2d, is much larger than any of the others; its divisions are bilateral and more numerous than in the corresponding cells of the other quadrants. This difference seems to be due to a shortening of the development and a consequent precocity in the segregation of materials destined to form the principal organs of the body. There is evidence, as will be shown later, that essentially the same organs develop from the cell 2d in Unio and Crepidula ; the difference therefore in this case is not one of material substance or destiny but rather a time difference according to which the development of 2d in Unio is compressed into a much smaller number of cleavages than in the case of Crepidula.

2. *The Third Quartette.*

All that has been said of the difficulties of tracing the cells of the second quartette is true in still greater degree of those of the third. The early divisions of this quartette are much slower than those of the second, and there are no distinguishing marks by which the cells may be known. I have therefore been unable to trace this quartette beyond the stage in which it gives rise to six cells in each quadrant, or twenty-four cells in all.

The first division of this quartette occurs at the stage when there are twenty-nine cells present. Before the division has been completed in all the quadrants the first quartette has divided twice and the second three times. The cleavage is not simultaneous in all the quadrants, the order of division being 3d, 3c, 3b, 3a (Figs. 25–28). The direction of the cleavage is nearly radial, though after the cleavage has

occurred it is seen to be plainly laeotropic in 3a, 3b, and 3c and dexiotropic[1] in 3d, *i.e.*, the cleavage is nearly bilateral on the posterior side of the ovum ; it is not purely bilateral, because the lower division product, $3c^2$, lies nearer the mid line on the right side than does the corresponding cell, $3d^2$, on the left, Figs. 29–35.

This is but another illustration of the fact that bilaterality first appears on the posterior side of the egg, that it is due to the change in direction of the cleavage of one out of four cells, and that it is not perfect when it first appears, but is merely a deviation from the spiral type toward the bilateral. In this case also, as in every other, the oblique character of the cleavage is much more pronounced after the daughter cells are formed than during the nuclear division.

By this first cleavage of the third quartette there is formed an upper and a lower cell in each quadrant. The lower cell ($3a^2$, etc.) is a little smaller than the upper ($3a^1$, etc.).

The next division of these cells occurs at the stage shown in Figs. 36 and 38. The upper cells of this quartette on the posterior side of the egg ($3c^1$ and $3d^1$) divide before the others and in a bilateral manner, Fig. 36. A little later, Fig. 38, the corresponding cells on the anterior side of the egg ($3a^1$ and $3b^1$) divide in a dexiotropic direction. There is now one lower cell and a right ($3a^{1.1}$, etc.) and left ($3a^{1.2}$, etc.) upper cell of this quartette in each quadrant.

Next the lower cell divides into right ($3a^{2.1}$, etc.) and left ($3a^{2.2}$, etc.) halves in each quadrant, Fig. 43 and Diagram 7. This division is slightly laeotropic in the anterior quadrants but bilateral in the posterior ones.

The outer products of this division on the posterior side of the egg ($3c^{2.1}$ and $3d^{2.1}$) then divide bilaterally into upper and lower products, $3c^{2.1.1}$ and $3c^{2.1.2}$, $3d^{2.1.1}$ and $3d^{2.1.2}$, Fig. 45.

About the same time the outer upper cells in the posterior quadrants ($3c^{1.2}$ and $3d^{1.2}$) divide into upper and lower products, ($3c^{1.2.1}$ and $3c^{1.2.2}$, $3d^{1.2.1}$ and $3d^{1.2.2}$) Figs. 44 and 45.

[1] The cleavage of this cell is not always reversed, for in some cases the division of the nucleus may take place in the usual, *i.e.* laeotropic, direction, and the daughter nuclei may lie in this position relative to each other and yet the cell body may show reversal of cleavage, *e.g.*, $3d^2$ Fig. 35.

Then the upper cells of the anterior quadrants ($3a^{1.1}$, $3a^{1.2}$, $3b^{1.1}$, $3b^{1.2}$) divide in a dexiotropic direction into the cells $3a^{1.1.1}$ and $3a^{1.1.2}$, $3a^{1.2.1}$ and $3a^{1.2.2}$, etc.

There are thus formed two upper, two middle, and two lower cells in each of the quadrants A and B, and two upper, one middle, and two lower cells in each of the posterior quadrants, C and D (see Diagram 8). These facts are brought together in the following tables, giving the lineage of 3a and 3d ; 3b is precisely like 3a, 3c like 3d.

Cell Stage	34	60–77	109
3a	3a' Upper	$3a^{1.1}$ Right	$3a^{1.1.1}$, $3a^{1.1.2}$ (Middle)
		$3a^{1.2}$ Left	$3a^{1.2.1}$, $3a^{1.2.2}$ (Middle)
	$3a^2$ Lower	$3a^{2.1}$, $3a^{2.2}$	
1	2	4	6
3d	3d' Upper	$3d^{1.1}$ (Inner) (E), $3d^{1.2}$ (Outer)	$3d^{1.2.1}$, $3d^{1.2.2}$
	3d² Lower	$3d^{2.1}$ (Outer), $3d^{2.2}$ (Inner)	
1	2	4	5

I have never seen the cells $3c^{1.1}$ and $3d^{1.1}$ divide, though they become quite large and the corresponding ones $3a^{1.1}$ and $3b^{1.1}$ do divide at the stage shown in Figs. 38 and 40. Every division of 3d and 3c is bilateral, except the first, which is transitional between the spiral and the bilateral. During this time the only other cells of the ectoblast which show bilateral cleavage are the tip cells in two of the arms of the cross, *viz.*, $2c^{1.1}$ and $2d^{1.1}$.

This is the history of the third quartette as far as I have followed it. It is particularly notable, in that bilateral symmetry within the ectoblast first appears in these cells, and that while all the other cells are dividing spirally the cells of this quartette in the two posterior quadrants always divide bilaterally.

Comparisons.

Blochmann ('81) has described the first division of the third quartette in Neritina (according to his designations the cells are a_2, $a^{IV}{}_2$, b_2 and $b^{IV}{}_2$, etc., but following the modification which I have suggested, they are, in the nomenclature of this paper, $3a^1$ and $3a^2$, $3b^1$ and $3b^2$, etc.). The cells lie closely pressed into the furrows between the macromeres; their first cleavage is apparently radial, and the lower cell product ($3a^2$, etc.) is smaller than the upper (Blochmann's Figs. 51 and 53). In passing, I merely call attention to the fact that this cleavage closely resembles the first division of the third quartette in Crepidula.

Blochmann gives no further history of these cells in Neritina, but his Fig. 56 shows what I believe is the second division of the third quartette. If my interpretation of this figure, which is given in Diagram 12, *b*, is correct, the upper cell in each quadrant ($3a_1$, $3b_1$, $3c_1$, $3d_1$) divides bilaterally in each case, giving off a smaller cell toward the mid line of the embryo. This division is in most regards like the corresponding one in Crepidula, but with this interesting point of difference: in Neritina all the cells of this quartette, on the anterior as well as on the posterior side, divide bilaterally. As in Crepidula, bilaterality appears most marked in the cells of the third quartette, but it is characteristic of all the cells of the quartette, and not merely of those of the two posterior quadrants.

The further history of these cells cannot be followed in Blochmann's figures. The following table presents a summary of the cleavages of the third quartette in Neritina:

3a	$3a^1$ (Upper)	$3a^{1.1}$ (Inner)
		$3a^{1.2}$ (Outer)
	$3a^2$ (Lower)	

The divisions of the third quartette in Umbrella are wonderfully like those in Crepidula. Heymons describes the direction of the first division as follows (p. 253): "Die Spindeln sind rechtwinkelig zur Dorsoventralachse des Eies gestellt," *i.e.*, as

his figures show, the spindles are placed radially. The lower cell ($3a^2$, etc.) in each case is smaller than the upper ($3a^1$, etc.), just as in Crepidula. After their formation, the lower derivatives move in a laeotropic direction, and the cleavage might therefore be considered as spirally dexiotropic. (The direction of this cleavage is not clearly marked in Heymons' figures, and, judging by his Figs. 8 and 12, it seems to me that the cleavage of 3c and 3d might be considered laeotropic. If this is really a dexiotropic cleavage, it is another violation of the alternation of cleavages, since the preceding cleavage was dexiotropic.)

The next division of this quartette is peculiar, and closely resembles the same cleavage in Crepidula. At the 38-cell stage, the cells $3c_1$ and $3d_1$ divide before the cells $3a_1$ and $3b_1$, and in a bilateral manner. Exactly this same thing happens in Crepidula. In Umbrella, the two products of this division lying nearest the mid line are the "Exkretzellen," or protoblasts of the larval excretory organs, and are designated E and E_1 by Heymons. The cells formed by this division ($3c^{1.1}$ (= E) and $3c^{1.2}$, $3d^{1.1}$ (= E_1) and $3d^{1.2}$), are large and clear; they have the same characteristics in Crepidula.

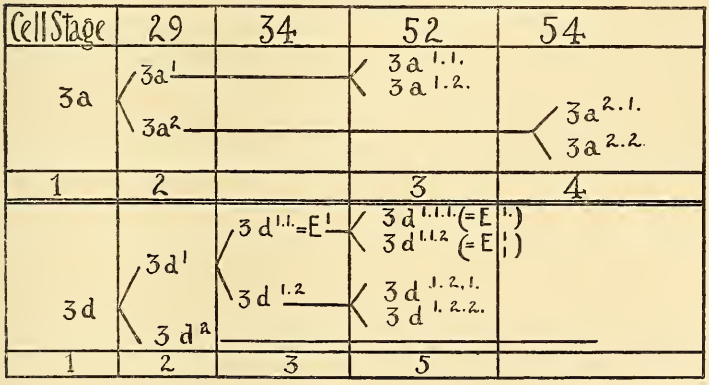
The corresponding divisions in the anterior quadrants, A and B, occur much later, *viz.*, at a stage when there are 52 cells present, and the cleavage is slightly laeotropic. In Crepidula, this cleavage occurs soon after the divisions of the posterior quadrants; and the spindles are nearly horizontal, as they are in Umbrella, but slightly dexiotropic instead of laeotropic. (From the position of the two cell products after the division, as shown in Heymons' Figs. 19 and 20, I should be inclined to consider this cleavage as slightly laeotropic, almost bilateral, in Umbrella.)

At the next cleavage there is a slight disagreement between Umbrella and Crepidula. According to Heymons the two upper cells in the posterior quadrant ($3c^{1.1}$ (= E) and $3c^{1.2}$, $3d^{1.1}$ (= E_1) and $3d^{1.2}$) divide in nearly a vertical direction, giving rise to two small cells on each side of the mid line, which lie alongside the lower cells $3c^2$ and $3d^2$. A little later the cells $3a^2$ and $3b^2$ divide as they do in Crepidula, but $3c^2$ and $3d^2$ do not divide. In Crepidula, on the other hand, all the lower

cells ($3a^2$, $3b^2$, $3c^2$, $3d^2$) divide at nearly the same time, and soon after, the outer upper cells in the quadrants C and D ($3c^{1.2}$ and $3d^{1.2}$) divide in nearly a radial direction, while $3c^{1.1}$ and $3d^{1.1}$ do not divide. In shape and position the cells resulting from these divisions are almost identically the same in Umbrella and Crepidula, though the method by which they arise is somewhat different in the two cases.

Heymons has observed two further divisions of his cells $c^{1.1.1}$ and $d^{1.1.1}$ (E and E1), *i.e.*, of the two upper cells in the quadrants C and D; but since I have not observed these divisions in Crepidula, I need not give a detailed description of them here.

The cleavage of the third quartette in Umbrella may be compared, at a glance, with the cleavage of the same cells in Neritina and Crepidula by means of the following table, which gives the lineage of 3a and 3d in Umbrella:



When we come to sum up the resemblances between Umbrella and Crepidula in the history of the third quartette, we find the same remarkable similarity which characterizes the cells of the other quartettes. Owing to the fact that bilateral symmetry appears in the posterior cells of this quartette (3c and 3d) the division of these two cells is highly peculiar, but all these peculiarities (at least as to the cell products, if not as to the method of their formation) are point for point exactly

alike in Crepidula and Umbrella. These resemblances are so minute and so long continued that they form a fitting climax to the many similarities which have been pointed out heretofore.

The divisions of the third quartette have not been followed by Wilson, Lillie, or Kofoid.

3. *Organs formed from the Second and Third Quartettes.*

As was indicated in another place (p. 114), all the organs of ectodermal origin lying posterior to the first row of velar cells are derived from the second and third quartettes, and a portion even of the foremost row of velar cells comes from the second quartette; nevertheless I have not been able, save in a few cases, to trace individual cells of these quartettes directly to the organs which they form. However, many of the organs of this region can be derived, in great probability, from certain *groups* of cells.

As is shown in Diagram 8, which is the latest stage to which the lineage of the whole ectoblast was traced, the first quartette occupies the apical region of the egg, and is surrounded by a broad belt of cells derived from the second and third quartettes. The cells of the second quartette lie at the ends of the arms of the cross, and approximately over the first and second cleavage furrows, which are still visible between the macromeres. The cells of the third quartette alternate with those of the second, and lie approximately halfway between the first and second furrows. In each quadrant the third quartette touches the first by only a single cell, but the cell group grows broader as it extends out toward the periphery of the egg; the second quartette is as broad where it touches the first quartette as at the periphery.

Since from this stage onward there is no extensive rotation of the cells around the egg axis, it is possible to locate some of the organs within certain of these cell groups. Other organs, particularly those near the middle of the ventral surface, cannot be traced even to these different cell groups with any degree of certainty, as I do not know what part the cells of the second and third quartettes take in the closing of the blastopore.

(a) *Blastopore, Stomodaeum, and Mouth.* — The ectoblast extends over the yolk from all sides at about the same rate, in consequence of which the blastopore closes near the middle of the ventral side. However, the *growth* of the ectoblast does not take place at the same rate in all directions, as is clearly shown by the fact that the apical cells do not lie opposite the blastopore at the time when it closes, but at the anterior end of the embryo, Fig. 65. The angular distance between the apical cells and the blastopore in this figure is less than 90° on the anterior side, while it is more than 270° between the same points in the opposite direction. This greater growth of the ectoblast on the posterior side of the egg must take place entirely in the cells of the second and third quartettes, since at this stage the growth of the first quartette is greater anterior to the apical cells than posterior to them. This unequal growth does not cause the blastopore to close more rapidly at its posterior side, but it does change the position of the apical pole, though the ventral pole remains fixed until after the closure of the blastopore.

At first the blastopore is not circular in outline; in fact, from the time when the germ layers are fully segregated, Figs. 42, 43, the cells of the third quartette in each quadrant lie slightly nearer the ventral pole than those of the second. This advance is somewhat increased in Fig. 47, so that the outline of the edge of the ectoblast is notched at four points, corresponding to the second quartette cells, and protrudes at the four intermediate points, which correspond to the third-quartette cells. In a later stage, Fig. 52, when the advancing edge of ectoblast can be seen from the ventral side of the egg, its notched character is still more apparent, the four notches forming the angles of a regular quadrangle. The angles of this quadrangle lie over the first and second cleavage furrows; at the posterior angle there is a broad recess in the lip of the blastopore, and within this recess are the four enteroblasts still uncovered by the ectoblast (see also Fig. 48).[1]

[1] The quadrangular form of blastopore has been described by Wilson in Nereis; and in that animal, as in Crepidula, the angles lie within cell groups derived from the second quartette, while the sides are formed by cells of the third quartette.

From this time on the blastopore closes from the sides more rapidly than from the anterior and posterior ends, and as a consequence the quadrangular shape is lost, and the blastopore becomes an irregular oval, Figs. 54, 57, 61, and then an elongated slit-like opening, Figs. 58, 60, 63. Finally both the anterior and posterior ends of the slit close, and there is left a narrow pore, Figs. 65, 66, 71, 72. Immediately around this pore there is a depression of the ectoblast, Figs. 65, 73, which is most extensive on the anterior and lateral sides The outlines of this depression become sharply marked, forming the fundament of the mouth; and its inner edges, especially the two lateral boundaries, turn inward as shown in Fig. 68, forming the fundament of the oesophagus or stomodaeum. For a brief period the stomodaeum is closed at its inner end, Fig. 88; but it soon opens again at the very point at which it closed, Figs. 90 *et seq.*, and thereafter remains in open communication with the cavity of the mesenteron. It is at first very short, Figs. 90, 91, but later becomes a long tube, Figs. 92, 93, 95. When it first begins to elongate, it is directed anteriorly from the mouth-opening to the mesenteron, so that it opens into the anterior part of that cavity, Fig. 92. In later stages, with the growth of the foot, the expansion of the shell gland, and the enlargement of the whole region posterior to the velum, the mouth-opening is pushed farther and farther forward, the yolk cells are shifted backward, and the whole direction of the stomodaeum is reversed, so that it runs posteriorly from the mouth-opening to the mesenteron, Figs. 78, 93, 95. Finally, with the greater development of asymmetry the inner end of the stomodaeum is moved slightly to the right, as shown in Fig. 82.

Throughout its entire length the stomodaeum is composed of columnar, ciliated cells, and along its posterior wall there is a double row of large clear cells, with cilia larger than usual, which is directly continuous with some large ciliated cells covering the median surface of the foot ; by the beating of these cilia the nutrient fluid surrounding the embryo is drawn into the mesenteron, Figs. 99, 104, 105.

(b) *The Posterior Growing-Point.* — At first the cell divisions on the posterior side of the egg are less frequent than on

the anterior side, Figs. 49–55. But, though the divisions are slow, the cell growth is rapid, in consequence of which the cells posterior to the transverse arms of the cross become enormous in size. The cells on the anterior side of the egg divide rapidly, but the total growth is less than that of the cells on the posterior side ; consequently the whole apical pole is shifted forward, Figs. 49–55.

Afterwards, at the extreme posterior end of the embryo, cell divisions begin, and proceed so rapidly that in a very short time there are more ectoderm cells on the posterior than on the anterior side, Fig. 64. The region of greatest activity lies just ventral to the future shell gland, and almost immediately over the mesoblastic teloblasts. Radiating from this region are more or less regular rows of cells, Figs. 64, 65, which are particularly well marked on the ventral surface. I have not been able to identify constantly any ectoblastic teloblasts, though in many eggs there are three or four large ectoblast cells lying between the mesoblastic teloblasts, from which many of the cell rows radiate. Two of these cells are shown at the extreme end of the embryo in Fig. 65. They are large cells with clear protoplasm, and from their position and character I believe that later they become the ciliated anal cells, which are shown in Figs. 78, 95.

The cell rows mentioned are much more pronounced in some eggs than in others. In a few cases they seem to cover the whole posterior end of the embryo, though in general they could be distinguished only on the ventral side. Here they run forward almost to the mouth as a series of branching, irregular rows, Fig. 65, and include the whole region which ultimately becomes the foot.

The cells from which these rows radiate lie on the mid line between the mesoblastic teloblasts, and must therefore be descended from the cell 2d. This cell also gives rise to a posterior growing-point in the annelids and in Unio, from which, in the case of the former, the ectoblast of the trunk is largely, perhaps entirely, derived, while in the latter the shell gland and foot are formed from the derivatives of this cell. The shell gland and foot in Crepidula are evidently formed from this

same cell. I do not doubt that this posterior region of teloblastic growth has essentially the same origin and destiny in Nereis, Unio, and Crepidula; and, if so, it follows that the cell 2d in Crepidula is really like the "first somatoblast" in Nereis and Unio, although its earlier divisions are very different. This difference, as I have already explained, is probably in the main a time difference, being due to the shortening of the history of this cell in the annelid and lamellibranch.

(c) *The Velum.* — The velum can first be distinguished as a row of small polygonal cells running across the ventral surface of the embryo immediately posterior to the apical plate and some distance in front of the blastopore, V^1, Figs. 65–67. These cells can be traced out to the sides of the embryo, where they turn forward and dorsalward, and finally become wholly indistinguishable from the surrounding cells. This row can be recognized in the velum throughout all the further development. It forms the most anterior row of velar cells, and ultimately becomes the most important part of the velum.

Just posterior to this first row is a second, V^2, which is composed of larger cells and is less distinct than the first row. The median portion of this second row can be recognized in all the older stages, Figs. 76, 79, 81, 82, as two or more large cells with clear protoplasm and vesicular nuclei; its lateral portions are not clearly marked.

The cells of these two rows are not ciliated at first, and can be traced only by their form and position. The long velar flagellae which they afterward bear do not develop until a late period, but from the time when the blastopore closes until these flagellae appear the embryo swims about in the egg capsule by means of the short cilia which cover the cells of the apical, dorsal, and pedal cell plates.

The median portion of the first row arises from the cells which lie just beyond the ventral end of the apical plate. These cells are in all probability $2b^{1.2.2.1.1}$ and $2b^{1.2.2.1.2}$. One of these cells is shown dividing in Fig. 71. In Fig. 72 a transverse row is formed from these cells which is plainly the first row of velar cells. It will be remembered that the apical plate is formed

from seven cells of the anterior arm of the cross, and that the terminal cells of this arm are probably thrown away.[1] Therefore the cells lying immediately beyond the anterior arm are the ones from which this median ventral portion of the first velar row comes. These cells may be traced back to a single one, $2b^{1.2.2.1}$, Figs. 46, 47, which lies at the end of the anterior arm. In this same position two cells are found a little later, Fig. 50, which have evidently come from this single cell by equal cleavage ; these cells are therefore $2b^{1.2.2.1.1}$ and $2b^{1.2.2.1.2}$. At a later stage they increase to four, Figs. 56, 71, and finally to six in Fig. 72.

The portion of the first velar row lateral to these six cells is evidently derived from the anterior turret cells, $1a^2$ and $1b^2$. These cells are shown undivided in Fig. 49, while in Fig. 50 they have divided bilaterally into the cells $1a^{2.1}$ and $1a^{2.2}$, $1b^{2.1}$ and $1b^{2.2}$. They are characterized by having clear protoplasm and large nuclei, and can be recognized for a considerable period lying just in front of the terminal cells of the right and left arms of the cross, and on each side of the median velar cells, Figs. 50–56.

The earliest figure which shows the protoblasts of the first velar row in position is Fig. 50 ; there are here two median and four turret cells, forming a series of six cells surrounding the cross on its anterior side, and extending from tip to tip of the transverse arms. In Fig. 56 they are shown increased to eight by the bilateral division of the median cells, and in Fig. 62 to ten or twelve. The terminal cells of the transverse arms of the cross divide, forming a row of four cells across the end of each arm, Fig. 56, and it is probable that these also must be added to the velar cells already described, making in Fig. 50 ten velar cells, in Fig. 56 sixteen, and in Fig. 62 at least eighteen or twenty. These cells belong to the first velar row only, and they extend from the mid-ventral line about two-thirds of the way around toward the mid-dorsal line. During all this time the posterior turret cells remain undivided, and the velar row ends dorsally against these cells.

It is probable that the mid-ventral portion of the second velar row, V^2, is derived from the cell which I have identified

[1] See Note p. 204.

provisionally as $2b^{2.2}$, and which lies just beyond the median cells of the first row, Figs. 56, 69, 70. I have not been able to determine whether any part of the second row arises by subdivision of the cells of the first; if not, this row may include a few cells of the third quartette ($3a^{1.1.1}$ and $3b^{1.1.1}$, Fig. 56) at the points opposite the anterior turrets.

These are the only velar cells whose origin I have been able to determine with any degree of probability; even in the case of these I recognize that there is an element of uncertainty, since the lineage was not followed cell by cell to a later stage than Figs. 47 and 48. However, I hold it highly probable that my identification of the velar cells in Figs. 50 and 56 is correct, and the identification in the later stages, Figs. 65 *et seq.*, is only a little less probable.

Several irregular rows of cells intervene between the first row of velar cells and the mouth, and in the latest stages figured several rows are seen running posterior to the mouth. All of these cell rows can be traced outward to the sides of the embryo, and all of them are ultimately ciliated and form part of the velum. Most of these cell rows could not have come from the first and second velar rows, and they must therefore have been derived from cells lying still farther away from the apical pole.

Thus the preoral velum is composed of a few cells of the first quartette, many of the second, and possibly a few of the third. It consists of many rows of cells, more or less regular in arrangement, extending from the first velar row in front to the edge of the mouth behind, Figs. 79, 81, 82. Of course the postoral velum must be composed of still more remote cells of the second and perhaps even of the third quartettes.

From the time of their appearance, Fig. 65, the first and second velar rows are slightly curved forward on the ventral mid line. In later stages, Figs. 76 *et seq.*, when, with the development of the foot and shell gland, the mouth is moved forward from the middle of the ventral face, this middle portion of the velum is carried still farther forward and at the same time the lateral portions of these velar rows are elevated above the general level and finally drawn out into a pair of

lobes which curve backward and downward on each side of the mouth, Fig. 79. Because of these opposite movements of the median and lateral portion of the preoral velum, but chiefly through the backward and downward growth of the lateral portions, the velum, when seen in apical view, bears a deep sinus on the ventral mid line, Figs. 79, 82.

In these later stages, Figs. 76 *et seq.*, the first velar row forms a slight ridge across the ventral mid line just posterior to the apical cell plate, which is particularly well marked because composed of a single row of small cells with densely staining nuclei which are bounded in front by the very large cells of the apical plate and behind by the large cells of the second velar row, Figs. 78, 79. Behind the cells of the second row and on the very edge of the mouth-opening is a third well-marked row, consisting like the first of small cells with densely staining nuclei, Figs. 79, 81, 82. This row can be traced laterally to the place where it joins the first row to form the margin of the velar lobe. At this point it bears a pair of prominences which ultimately become the tentacles, Fig. 81, T ; these structures therefore are formed in the preoral velum, in fact in the second cell row of the prototroch. In later stages they lie over the cerebral ganglia.

On the mid line these rows of velar cells are raised but a little above the general level, but laterally they are borne on the margins of the very prominent velar lobes. Cross sections of these lobes show one row of large rounded cells, which forms the extreme margin of the lobe and bears the long velar flagellae. On each side of this are one or more rows of large crescentic-supporting cells, Figs. 103–105. Similar cells have been described by Patten ('86) as present in Patella.

The postoral velum is not well defined until the last stage shown in the drawings, Figs. 81, 82, though somewhat irregular rows of nuclei can be seen crossing the body posterior to the mouth in stages as early as Fig. 76. In these later stages a ridge of cells runs out from the posterior edge of the velar lobe and can be distinctly followed to the ventral mid line of the foot. This is the postoral ridge of velar cells, and it runs

around the margin of the velar lobe on the posterior side, being separated from the preoral ridge by a shallow ciliated groove. On the ventral surface these ridges are widely separated, the preoral lying some distance in front of the mouth, as the postoral is some distance behind it. As shown in Figs. 81, 82, the postoral ridge crosses the foot posterior to the large ciliated cells which lie just behind the mouth, and on the mid line the ridge from each side turns backward and ends in a median row of ciliated cells. The whole area between the anterior and posterior ridges is clothed with a coat of fine cilia.

Laterally the postoral ridge grows less prominent, and in sections of the velar lobes taken about halfway between the dorsal and ventral surfaces, the postoral velum is merely a series of columnar, ciliated cells running around the posterior margin of the velar lobes, Figs. 103–105.

Passing up toward the dorsal side, the velum divides on each side of the embryo into two branches, Figs. 78, 80. The posterior branch, which is much smaller than the anterior one, continues up over the dorsal surface posterior to the head vesicle, being incomplete, however, on the dorsal mid line. The anterior and larger branch turns forward in a sharp curve on each side of the body, and ends abruptly on each side of the apex, Figs. 80, 82. The cells lying between the two branches on the dorsal surface are the large ciliated cells of the posterior cell plate.

At the point where the branching occurs, one large cell is found directly in the angle between the two branches, Figs. 77–80. This is, I believe, the posterior turret cell of each side. I could not determine whether the turrets contribute anything to the formation of the velum on the dorsal side as they do on the ventral. In one sense they lie within the velum, as does the whole posterior cell plate, being bounded in front by the anterior and behind by the posterior branches, but in any case the foremost row of the velum lies nearer the apex on the dorsal side than it does on the ventral, since it runs on the apical side of the posterior turret cells. From its position relative to the apical organ and the large cells of the posterior plate, it is probable that this anterior branch of the

velum follows the posterior edge of the right and left arms of the cross from tip to base.

I have not observed any cilia on the posterior branch, and believe that it is not functional as a locomotor organ; the anterior branch, on the other hand, is clothed with long velar flagellae, and in later stages grows more and more prominent, forming the dorsal part of the velar lobe.

The posterior branch seems to be a continuation of the entire velum rather than of either the preoral or postoral portions; the velum is therefore double on the dorsal side of the embryo. The posterior branch occupies the position of the velum in Ishnochiton and of the prototroch of the annelids, and I interpret the fact that it bears no cilia as indicating that it is a phylogenetic remnant of the ancestral velum. The anterior branch, on the other hand, is a new aquisition not represented in Chiton, nor even in the more primitive gasteropods.

It is now known that there is a postoral band of cilia in quite a large number of molluscan larvae. Brooks ('76) was, I believe, the first one to discover this band. Since then Haddon ('82) has shown that it is present in some Nudibranchia; Hatschek ('80) has described it and an adoral band, together with the usual preoral band, as present in Teredo; and McMurrich ('85) has described a postoral band of cilia as present in the veliger of Crepidula.

Judging from position and structure there can be little doubt that the anterior ciliated ridge in all these cases is homologous with the *preoral ciliated band* in annelids, the posterior ridge with the *postoral band*, and the ciliated groove with the *adoral band*.

In its fully formed condition at the beginning of larval life, the velum of Crepidula is a very large and an extremely complex structure, consisting of many rows of ciliated cells running around the margin of the wheel-like lobes which are borne on each side of the head vesicle of the larva. The locomotor flagellae are very long and powerful, and their movements indicate some kind of nervous control. The entire margin of each lobe is surrounded by many regularly arranged pigment spots, which are beautifully colored, one row being a delicate green,

another a faint red, and still another sepia or black. Each velar lobe contains many stellate mesoblast cells, and the whole structure is highly irritable and contractile.

This structure is much larger and more complex than the annelid prototroch; and perhaps nowhere, with the possible exception of the echinoderms and Enteropneusta, is there a larval locomotor organ which will compare in size and complexity with the velum of many gasteropods.

Comparisons.

Reference has already been made to the origin of the velum in Neritina (p. 94). Blochmann was able to trace the tip cells of the transverse arms ("Urvelarzellen") to this structure, but he does not indicate what other cells enter into it. Of these velar cells he says (p. 162): "Schon während das Ektoderm anfing sich nach der ventralen Seite hin auszubreiten, sind die Zellen *vz* und vz_1 an die beiden Seiten gerückt, und in einer dieselben verbindenden Zellreihe werden dieselben lichtbrechenden Körnchen bemerkbar (Fig. 66), wodurch eine weitere Ausbreitung des Velums angedeutet wird. Dasselbe erscheint jedoch noch nicht kontinuirlich, sondern von den ursprünglichen Velarzellen *vz* und vz_1 ausgerechnet sind jederseits nur zwei oder drei Velarzellen sicher zu erkennen. Auch ventral ist das Velum noch nicht geschlossen."

As I have already indicated, it is very probable that these same cells form the lateral portions of the velum in Crepidula. I was not inclined to accept this view at first, because on the anterior side the first velar row lies beyond the tip cells of the anterior arm[1] and because the tip cells of the right and left arms seemed at first sight very far removed from the velum. Accordingly, I said in my first preliminary ('91): "In Crepidula it seems that no part of the transverse arms forms the velum." However, a more prolonged and careful study of the velum shows that these terminal tip cells, increased to four on each side, very probably form the lateral portions of the first velar row. There is thus the most exact agreement between these two animals in the origin of this portion of the velum.

[1] See Note p. 204.

As to the origin of other portions, no comparisons can be drawn since Blochmann has made no further observations on this point. The turret cells in Neritina are very small, and have not divided up to the last stage in which they can be recognized, and one cannot tell from Blochmann's figures whether they form any part of the velum or not. His figures would indicate, though they would by no means establish this point, that the velum does not branch dorsally as in Crepidula.

Heymons did not observe the origin of the velum in Umbrella. However, the following statement quoted from his work (p. 278) shows that in this animal the velum is the same in its general appearance, and must occupy essentially the same position as in Crepidula: "Die vorderste Partie des Eies, die dem früheren animalen Pol entspricht, wird von hellen grossen Ektodermzellen bekleidet. Dieser ganze Theil setzt sich bald noch schärfer als in früheren Stadien von der übrigen Masse des Eies ab, und zwar geschieht dies besonders durch das Auftreten des Velums. Letzteres beginnt sich gleich nach dem Verschluss des Gastrulamundes zu zeigen und besteht anfänglich aus einigen hellen und körnchenreichen Ektodermzellen, die sich später aneinander legen und dann in einer kontinuirlichen Reihe rings um den Embryo herum ziehen. Der von ihnen umschlossene Bezirk ist als Velarfeld zu bezeichnen. Die Mitte desselben fällt mit dem früheren Centrum des animalen Poles zusammen, welches, wie oben erwähnt wurde, mitsammt den Richtungskörpern an das Vorderende des Embryonalkörpers gelangt war." Heymons observed the first division of the four turret cells, but he did not follow them to their destination.

There is no velum or prototroch in Unio, and consequently we need not be surprised to find certain cells which enter into the velum in Crepidula diverted to other uses in that animal. Thus Lillie finds that the cells $2a^{1.1}$, $2b^{1.1}$, and $2c^{1.1}$, which are velar cells in Crepidula, assist in forming the larval mantle of Unio. He records two divisions of each of the turret cells, but did not determine their destiny.

Wilson, in his work on Nereis, first established the exact cell origin of the prototroch among the annelids. In this animal it

is formed entirely from the four turret cells, or trochoblasts, as Wilson appropriately calls them. Each of these cells divides twice, forming in all sixteen cells, twelve of which compose the prototroch. By the growth and division of these cells the tip cells of the cross are forced into a position below the prototroch, and are called by Wilson "post-trochal cells." Though they take no part in forming the prototroch, they lie but one cell below it, and may perhaps be considered as having been crowded out of the trochal series by the rapid growth and early division of the trochoblasts.

Mead ('94) has found that in Amphitrite and Clymenella the same cells form the prototroch as in Nereis. Each of these trochoblasts divides twice, as in Nereis, and all sixteen of these cells enter the prototroch. "Later the prototroch is completed by the addition of nine more cells from the 'second generation of micromeres' in quadrants A, B, and C respectively." That these additional cells of the second quartette are the same, at least in part, as the velar cells of Crepidula is shown by the further statement made by Mead that "almost the entire substance of $a^{2.1}$ (2a1) and $c^{2.1}$ (2c1) enters into the prototroch."

Remembering the many points of difference between the fully formed velum of the gasteropod and the prototroch of the annelid, it is most interesting and instructive to find such essential agreement in origin between the two. In fact, it may be truly said that they are even more alike in origin than in final structure.

(d) *The Shell Gland.* — This characteristic molluscan organ appears late in development in the case of Crepidula, Figs. 74 *et seq.* From its position on the mid line and at the posterior end of the embryo it is probable that it comes from the group of cells derived from 2d.

It is formed in the first instance by the very rapid multiplication of cells in a limited region of the ectoblast. These cells are densely packed together, so that in surface views of the egg only the nuclei can be recognized. At the centre of this proliferating area the nuclei are smallest and most numerous, and they grow successively larger from the centre toward the

periphery, Fig. 74. This proliferating area then invaginates, forming at first a shallow depression, and later the edges of this depression arch over until they nearly meet in the centre, Figs. 75, 77, 92. A short time after this the edges begin to extend in every direction, the invagination becomes shallower and broader, and at the same time a thin cuticle, which is the first trace of the shell, appears over the surface of the invaginated cells, Figs. 78, 95, 104, 105. While the shell gland is comparatively small, these cells are columnar, Figs. 92, 95, but as it increases in size they become extremely flat and thin, so that it is scarcely possible to see them even in sections, Figs. 103, 105. The cells at the margin of the shell gland, however, are columnar, and it is from these that the growth of the shell takes place.

Owing to the shifting of the posterior end of the embryo toward the ventral side, as shown in Figs. 80, 93, 95, the shell which was at first on the postero-dorsal area comes to be located entirely at the posterior end of the embryo, which now appears truncated, Figs. 80, 93, 95. The margin of the shell gland then extends forward on the left side much more rapidly than it does on the right, and at the same time the whole posterior part of the embryo is pushed over to the right.

In both Neritina and Umbrella the shell gland forms on the postero-dorsal surface of the embryo, but in neither case has its cell origin been determined. In Unio it is derived from the cell 2d, or the "first somatoblast," and, as we have seen, it probably comes from the same cell in Crepidula.

In Fulgur the invagination of the shell gland occurs at an early period, when the ectoblast has extended but a short distance over the yolk. Its early appearance seems to have misled McMurrich ('86), who regarded it as an invagination of unknown significance, but of very general occurrence. Except for its early appearance it is in all respects similar in origin and development to the shell gland of other gasteropods.

(e) *The Foot.* — Immediately after the formation of the shell gland the foot appears as a single median protuberance on the ventral surface, Figs. 76, 77. At first the prominence is about as long in the antero-posterior diameter as it is wide, but

in later stages it is much broader than long. On the median surface of the foot there are several large ciliated cells which resemble the apical and dorsal cells, and which I have therefore called the pedal cell plate. All the rest of the foot is covered by a columnar epithelium of ectoderm cells. When it first appears it is about equally prominent over its whole surface, Figs. 76, 77, but in the course of further development the posterior part becomes much more prominent than the anterior. The foot is, as it were, tipped up on its anterior edge by being crowded forward from behind. This forward tilting continues, as shown in Figs. 80–82, until the foot, instead of lying posterior to the mouth as it did at first, lies ventral to it. At an early stage the ectoderm forming the foot separates from the yolk beneath, and the cavity thus formed becomes traversed in every direction by mesoderm cells.

About the time that the supraoesophageal ganglia first appear the otocysts arise as small invaginations of the ectoderm on each side of the foot. They are at first open pits, which gradually close, forming vesicles the outer walls of which lie in the layer of ectoderm covering the foot, Fig. 100. At first the vesicle is quite small, and the cells surrounding it are cuboidal, but in later stages it increases in size and its walls grow thinner, Figs. 80–82, 105; at the same time its outer wall separates entirely from the ectoderm covering the foot and the vesicle comes to lie entirely within the cavity of the foot. The cerebro-pedal connectives end directly against the otocysts, and a small strand of cells, the origin of which I have not determined, connects the otocysts of the two sides.

In the oldest embryo figured the foot bears a thin cuticular operculum over its posterior surface. At a still later stage it becomes much more prominent and is triangular in outline, the apex being directed ventralward and forward. A very great number of cells which stain deeply and are probably gland cells are distributed quite uniformly in its epithelium.

As has been mentioned (p. 131), Lillie has found that the foot in Unio is formed from a portion of the ventral plate, which is derived from the first somatoblast, X (2d). I have called attention to the fact that in Crepidula the cell rows

which are formed on the ventral side anterior to the growing-point probably correspond to the ventral plate of Unio, and also, as in that animal, give rise to the foot. There is good reason to believe that these cell rows ultimately come from the cell 2d.

The cell origin of the foot is not given by either Blochmann or Heymons. From the latter's figures, however, it is evident that its place of origin and early history in Umbrella is essentially the same as in Unio and Crepidula.

In Patella (Patten, '86) and Fulgur (McMurrich, '86) the foot is said to arise as two lateral swellings which subsequently fuse together on the ventral mid line. Although it is single in its origin in Crepidula, the row of large transparent cells along its median surface gives it the appearance of being double, especially in the large embryos of C. convexa and C. adunca ; however, careful study of profile views and of sections shows that it is not double, but is a single median protuberance.

(f) *The External Excretory Cells.* — On each side of the embryo, just posterior to the velum and dorsal to the foot, several of the ectoderm cells swell up and gradually lose their nuclei and cell boundaries, Fig. 78 ; the cells become vacuolated, and the vacuoles are filled with small granules which stain deeply. Later the several vacuoles seem to flow together into one or more large ones, Figs. 80, 81. In the early stages these cells form a part of the ectodermic layer, but as the embryo grows older they grow more prominent, and the whole mass is constricted at the base, so that it becomes pear-shaped, the narrower end being attached to the embryo and the larger end being distal, Figs. 81, 104. The surrounding ectoderm cells crowd in at the neck of this constriction, and work their way entirely beneath these excretory cells. About the beginning of the free larval life many of the vacuoles with their granular contents disappear, and there is left on each side a clear, pear-shaped mass, which is attached for a time in the deep constriction posterior to the velum. Ultimately these structures appear to be pinched off completely. I have not observed them in the process of being cast off, but they suddenly disappear and leave no trace behind, except that one

sometimes finds one of these masses lying near an embryo, but wholly free from it ; I conclude, therefore, that they are thrown away.

When the embryos are stained with haematoxylin, the granular contents of the excretory cells stain dark carmine, while all the remainder of the embryo stains a royal purple, or dark blue. This carmine color, as is well known, can be produced by treating haematoxylin with weak acids, and the fact that these excretory cells stain a carmine color may indicate that they contain some acid secretion.

Heymons has traced with great care the history of the external excretory cells in Umbrella. They are derived from the cells $3c^{1.1}$ and $3d^{1.1}$. These cells divide, and then sink into the interior of the body and are overgrown by ectoderm cells. They are afterwards filled with brown concrement particles. Generally the right excretory cells develop, while the left do not.

These cells lie near the anal cells, at the posterior end of the embryo, and far removed from the velum and foot. I cannot, therefore, believe that they correspond in cell origin to the excretory cells of Crepidula, although $3c^{1.1}$ and $3d^{1.1}$ in Crepidula repeat in a most remarkable way all the peculiarities of the same cells in Umbrella, having been formed by bilateral cleavage, and being large, clear cells with vesicular nuclei. I have not been able to trace the cell origin of the excretory cells in Crepidula, and the possibility remains that they are derived from $3c^{1.1}$ and $3d^{1.1}$, as in Umbrella. If this be the case these cells must be displaced much farther forward than in Umbrella, possibly by the more active division of the ectoderm cells near the growing-point. But on the other hand it is possible, as is evidenced by the dissimilarity in their later history, that the excretory cells in Umbrella and Crepidula are not homologous, and that they do not have the same cell origin.

Heymons does not consider the differences between these external excretory cells of prosobranchs and opisthobranchs to be any serious objection to their homology. He says (p. 293): "Der Umstand, dass die äusseren Urnieren der Prosobranchier

paarig sind, wird keine Schwierigkeit machen, da die Entwicklungsgeschichte von Umbrella für eine ursprünglich paarige Anlage spricht. Auch die Lage des Organs am hinteren Körperende braucht noch kein Grund dagegen zu sein. Durch die Untersuchungen von McMurrich wissen wir, dass die äusseren Exkretionszellen der Prosobranchier ebenfalls bald etwas weiter vorn, bald etwas weiter hinten sich befinden." This difference in position seems to me, however, to be a very considerable one. In all prosobranchs these cells lie close behind the velum, while in Umbrella they are removed from that structure by almost the whole diameter of the embryo. Further, the fact that they sink into the interior in Umbrella would indicate that they are different from the excretory cells of prosobranchs.

Rabl ('79) believes that the so-called "primitive excretory cells" have no excretory function at all, but are merely a part of the velum. The fact, however, that in very many forms they are found to contain granules or crystals which have been seen to be extruded from the cells lends support to Bobretzky's ('77) idea that these cells really have an excretory function, and the fact that they are completely cast off in Crepidula would still more strongly support that view. That they are any portion of the velum, as has been maintained by Rabl and McMurrich, seems to me to be distinctly negatived by their general position behind that organ and their complete separation from it.

Bobretzky ('77) says that the ectoderm passes unbroken beneath these cells, while McMurrich ('86) believes that the excretory cells form a part of the layer of ectoderm covering the embryo, and that therefore they are not underlaid by ectoderm. In Crepidula, as we have seen, these excretory cells at first form a part of the general ectodermal layer, and are not underlaid by ectoderm; in later stages, however, the ectoderm forms a layer beneath them very nearly if not quite complete. If the conditions which prevail in Crepidula are general, it is probable that McMurrich based his conclusions on the study of younger stages, while Bobretzky was guided by the study of older ones.

In closing this section on the history of the ectomeres, I append a table, giving the number of cells in the various quar-

Number of Divisions in Different Quartettes.

	Cell Stage	8	12	16	20	24	28	32	36	40	44
	1. Quart.	4	—	8	—	—	—	16	16	20	24
Discocoelis	2. Quart.	0	—	4	—	—	—	8	8	8	8
	3. Quart.	0	—	0	—	—	—	4	4	4	4
	4. Quart.	0	—	0	—	—	—	0	4	4	4
	Cell Stage	8	12	16	20	23	29	32	36	38	42
	1. Quart.	4	—	8	—	12	16	16	20	20	22
Nereis	2. Quart.	0	—	4	—	5	5	8	8	9	10
	3. Quart.	0	—	0	—	2	4	4	4	4	4
	4. Quart.	0	—	0	—	0	0	0	0	1	2
	Cell Stage	8	12	17	20	22 24	27	32	36	38	39 46
	1. Quart.	4	4	8	—	8 —	9	10	—	10	10 16
Unio	2. Quart.	0	4	5	—	9 —	10	13	—	18	19 19
	3. Quart.	0	0	0	—	1 —	4	4	—	5	5 5
	4. Quart.	0	0	0	—	0 —	0	1	—	1	1 2
	Cell Stage	8	12	16	20	24	25 29	33	37	38	40 44
	1. Quart.	4	4	4	8	8	8 8	8	8	8	8 12
Umbrella	2. Quart.	0	4	4	4	8	8 8	12	16	16	16 16
	3. Quart.	0	0	4	4	4	4 8	8	8	8	10 10
	4. Quart.	0	0	0	0	0	1 1	1	1	2	2 2
	Cell Stage	8	12	16	20	24	25 29	30	34	38	42 44
	1. Quart.	4	4	8	8	8	8 12	12	12	12	12 12
Crepidula	2. Quart.	0	4	4	4	8	8 8	8	8	12	16 16
	3. Quart.	0	0	0	4	4	4 4	4	8	8	8 8
	4. Quart.	0	0	0	0	0	1 1	2	2	2	2 4
	Cell Stage	8	12	16	20	24	28	32	36	41	45 46
	1. Quart.	4	4	8	8	8	12	—	11	12	16 16
Neritina	2. Quart.	0	4	4	4	8	8	—	16	16	16 16
	3. Quart.	0	0	0	4	4	4	—	4	8	8 8
	4. Quart.	0	0	0	0	0	0	—	0	1	1 2
	Cell Stage	8	12	16	20	24	25				
	1. Quart.	4	4	—	—	8	8				
Planorbis	2. Quart.	0	4	—	—	8	8				
	3. Quart.	0	0	—	—	4	4				
	4. Quart.	0	0	—	—	0	1				
	Cell Stage	8	12	16	20	24	28	32	36	40	44
	1. Quart.	4	4	8	8	8	8	12	12	12	16
Limax	2. Quart.	0	4	4	4	8	8	8	8	12	12
	3. Quart.	0	0	0	4	4	8	8	8	8	8
	4. Quart.	0	0	0	0	0	0	0	4	4	4

COMPARISON OF LATER STAGES IN NEREIS, UMBRELLA, AND CREPIDULA.

	Cell Stage	48	52	56	58					
	1. Quart.	—	—	—	36					
Nereis	2. Quart.	—	—	—	12					
	3. Quart.	—	—	—	4					
	4. Quart.	—	—	—	2					
	Cell Stage	47	51	55	59	65	69	75	83 93	101
	1. Quart.	12	12	12	12	12	16	20	20 24	28
Umbrella	2. Quart.	16	20	24	24	24	24	24	32 36	36
	3. Quart.	10	10	10	12	18	18	20	20 20	20
	4. Quart.	5	5	5	7	7	7	7	7 9	9
	Cell Stage	47	52		58 60	64	68	77	88	111
	1. Quart.	15	15		15 15	15	15	19	23	26
Crepidula	2. Quart.	16	16		22 22	24	24	25	32	44
	3. Quart.	8	8		8 10	12	14	16	16	24
	4. Quart.	4	9		9 9	9	11	13	13	13

tettes at different stages for all forms with spiral cleavage which have been sufficiently studied. It will be seen that up to the 44-cell stage, beyond which it is impossible to carry the comparison in most cases, the first quartette divides most frequently in the annelid and the polyclade, the second and third quartettes in the mollusk, and the fourth quartette in the polyclade and mollusk. A comparison of Nereis, Umbrella, and Crepidula at the 58-cell stage shows still more plainly that the divisions of the first quartette are very rapid in the annelid, while those of the second and third quartettes are much more rapid in the mollusk.

While in general the number of cell divisions may be taken as a measure of the development, precocity in cell division does not always indicate precocity in differentiation ; *e.g.*, in the case of the first somatoblast (2d) the differentiation is much slower in the gasteropod than in the annelid, and yet the cell divisions are more numerous in the former than in the latter. Cell division is not always associated with differentiation, and therefore the measure of differentiation cannot always be determined by the number of divisions. In the cases compared above, however, there is no doubt that the more rapid

divisions of the first quartette in the annelid are associated with the more rapid differentiation of the upper hemisphere in that animal.

VI. History of the Mesomeres.

The origin of the mesoblast was treated in a previous part of this paper, and its history was traced up to the time when it is completely separated from the other germinal layers. The primary mesentoblast (4d) is formed at the 24-cell stage, but the complete segregation of its mesoblastic and entoblastic constituents does not occur until there are 65 cells present, of which eight are the descendants of 4d. Of these eight cells, four lie on each side of the mid line; the two posterior ones on each side are the enteroblast or intestinal cells, the two anterior ones are mesoblast cells, Figs. 42, 44, 46. These mesoblast cells, four in all, form the beginning of two bands, which ultimately extend about halfway around the egg.

1. *The Mesoblastic Bands.*

The posterior mesoblast cell is the teloblast, or "pole cell," of the bands. It is a large rounded cell, free from yolk granules, and, when stained, is rather darker than any of the surrounding cells. It is frequently seen dividing, and always so as to add new cells to the posterior ends of the bands. The anterior cell on each side (primary mesoblast) is the first purely mesoblastic cell formed. It is much smaller than the teloblasts, and has less affinity for stains. These two anterior cells divide soon after the teloblasts are formed, Fig. 42, usually across the long axis of the bands, but sometimes in the direction of that axis, Fig. 46.

The bands grow in length both by the addition of new cells at their posterior ends and by the subdivision of the cells already formed. They ultimately extend around the periphery of the egg from near the mid line behind to the first, or transverse, furrow on each side. In all the figures up to and including Fig. 53 these bands lie nearer the dorsal than the ventral side, but in all stages older than Fig. 53, they are nearer the ven-

tral side; this is due to the fact that they are carried down over the yolk cells with the overgrowing ectoblast. When first seen on the dorsal side, Fig. 42, etc., the teloblasts and their bands lie anterior to the enteroblasts. After they have moved around to the ventral side of the egg, with the general overgrowth of the ectoblast, the teloblasts lie posterior to the enteroblasts, Figs. 57 *et seq.*, although preserving the same position relative to the bands as before. This apparent change of position can be readily understood by comparing Figs. 51–54, in which an intermediate condition is shown.

Even in the later stages the nuclei of these mesoblast cells can be plainly seen just beneath the ectoblast, but it soon becomes impossible to recognize the cell boundaries, especially at the anterior ends of the bands. At the last stage in which the entire bands can be seen, Fig. 53, there are eight or nine cells in each. In older embryos the teloblasts and posterior ends of the bands may remain distinct, while only scattered nuclei can be recognized at the anterior ends, Figs. 57 *et seq.*; but ultimately the teloblasts and all traces of the bands disappear, Figs. 74 *et seq.*

2. *The Scattered Mesoblast Cells.* (*Larval Mesoblast.*)

In no case can the cells derived from the bands be traced anterior to the first, or transverse, cleavage furrow, nor over the ventral face into the lips of the blastopore. Yet in all stages from Fig. 60 onwards scattered mesoblast cells are abundant at the anterior end of the embryo and over the whole ventral surface, but particularly in the region of the blastopore. It seemed impossible that so many cells so widely scattered could have come in so short a time from the mesoblastic bands, and I was therefore led to look for another source of this scattered mesoblast, especially after the publication of Lillie's ('93) beautiful results on the double origin of the mesoblast in Unio. I was not able, however, to trace these scattered cells to their source in the relatively small eggs of C. plana and C. fornicata; but since the plates of this article were sent to the lithographer I have found in the large eggs of C. convexa and C. adunca, of

about the stage shown in Fig. 52, one additional mesoblast cell in each of the quadrants A, B, and C, lying immediately below the ectoblast cells at the angles of the quadrangular blastopore. As was shown on page 129, these ectoblast cells are derivatives of the second quartette; and since the additional mesoblast cells are derived from the overlying ectomeres, it follows that they have come from the cell groups 2a, 2b, and 2c, though I cannot give their exact cell origin. In the few cases which I have been able to examine, the additional mesoblast cell is formed first in quadrant A, and afterward one is formed in each of the quadrants C and B. In C and B they lie on the right (as seen from the vegetal pole) of the cells 4c and 4b, while in quadrant A the mesomere lies to the left of the cell 4a. In other words, these additional mesoblast cells are bilaterally placed in the quadrants A and C. There is no doubt that the scattered mesoblast above described comes from these additional cells.

These additional mesoblast cells forcibly recall the "larval mesoblast" of Unio. Like the latter, they are formed from the second quartette of ectomeres, they are not teloblastic in growth, but give rise to scattered mesoblast cells, and they seem to be concerned chiefly in the formation of unicellular muscle fibres, or myocytes,[1] which appear in the foot and in those larval organs, the head vesicle and velum. On the other hand, these cells differ from the larval mesoblast described by Lillie in the fact that they arise in three quadrants instead of in one only as in Unio, they appear at a much later stage, and they probably give rise to adult as well as to larval structures. These differences, however, are of secondary importance as compared with the resemblances mentioned, and I do not doubt that these cells correspond to the larval mesoblast of Unio.

The origin of larval mesoblast in three quadrants is most suggestive, since it points, as I believe, to a primitively *radial* origin of the mesoblast. From every point of view it seems probable that Crepidula represents a more primitive condition in this regard than Unio. The radial symmetry of the other layers is more complete in Crepidula than in Unio and is pre-

[1] I follow Lillie ('95), p. 38, in the use of this term.

served much longer. The asymmetrical origin of the larval mesoblast in Unio is probably associated with the extreme precocity of development which is shown in the very early differentiation of this and of so many other fundaments in that animal. In all cases which I have observed, the larval mesoblast cell in quadrant A, which is the only one found in Unio, is the first one formed in Crepidula.

It is a most interesting fact that the larval mesoblast in Crepidula arises in the three quadrants which have produced no other mesoblast, *viz.*, A, B, and C ; the quadrant D, which gives rise to the paired mesoblast, produces no larval mesoblast.[1]

Mesoblast is therefore produced in each of the four quadrants. In A, B, and C it is derived from the ectomeres of the second quartette ; in D, from the fourth quartette. In all cases the segregation of mesoblast in the cell 4d is associated with elongation of the body and teloblastic growth, even in such animals as lamellibranchs and gasteropods, which are not generally considered elongated animals. In more primitive forms there is probably no teloblastic growth, and consequently the mesoblast may arise in the same way in each quadrant, as is said to be the case among polyclades and ctenophores.

From these facts it is probable that the radial origin of mesoblast is to be considered a primitive character; its bilateral origin, a secondary one. In other words, the larval mesoblast is the more ancestral, and it might properly be called the *primary* or *radial mesoblast*, while that formed from 4d might be known as *secondary* or *bilateral mesoblast*.

Throughout embryonic life all the mesoblast is but scantily developed and exists for the most part as fusiform cells. These cells are most numerous in the foot, where they form the myocytes which traverse the cavity of the foot in every direction ; they are also found in considerable numbers in the velar lobes and in the head vesicle.

[1] Of course it is possible that this quadrant does give rise to larval mesoblast at a stage *much later* than that at which it arises in the other quadrants. The most diligent search, however, has so far failed to reveal it, and if larval mesoblast is ever produced in quadrant D, it must be so much later than its origin in the other quadrants as to deserve to belong to another series of phenomena.

Comparisons.

In Neritina well-marked bands of mesoblast are formed which have the same axial relations and general appearance as in Crepidula. In the latest stage shown by Blochmann in which these cells appear, Fig. 66, there are four cells in each band and the teloblasts are some distance removed from each other. As has been remarked in another place (p. 74), these cells are connected with each other by several small entoblast cells, Figs. 62, 65, which probably correspond to the enteroblasts of Crepidula, since they are said to form the intestine. As development proceeds the bands move farther down on the sides of the egg and are separated from the small entoblast cells. There is no suggestion in Blochmann's work of scattered mesoblast such as is found in Crepidula.

The formation and early history of the mesoblast bands in Umbrella were described on page 72. In the later stages Heymons has observed the dissolution of the bands and the nearly uniform scattering of their cells; at the same time the teloblasts disappear.

The group of small cells which lies between the teloblasts and corresponds in position, though not in origin, to the enteroblasts of Crepidula "probably gives rise to mesoblast cells which later are found on the outer surface of the intestine." A similar layer of mesoblast cells is found surrounding the intestine in Crepidula, Figs. 80, 81, though I have not supposed that it was derived from the cells lying between the teloblasts, *viz.*, E^1, E^2, e^1, e^2. The fact, however, that there is an exactly similar group of cells (the "secondary mesoblast") in Nereis which has exactly the same fate, and that in Unio also there is a similar group which, as Lillie believes, has the same destiny, is strong corroborative proof of the accuracy of Heymons' observations. In Nereis these cells lie uncovered at the posterior lip of the blastopore; they afterwards become pigmented and migrate inward, where they spread out upon the wall of the mesenteron. Lillie believes that essentially the same thing happens in Unio. In Crepidula this group of cells, four in number, which lies between the teloblasts gives rise, as I

believe, not to the mesoblast covering the intestine, but to the distal portion of the intestine itself.

Heymons did not observe a secondary origin of mesoblast in Umbrella, though he suggests that possibly in stages later than he has examined, ectoderm cells may migrate into the interior to form mesoderm.

VII. History of the Entomeres.

At the time when the mesoblast is completely separated from the entoblast the latter consists of the following cells :

Macromeres (Basal Quartette)	4
Smaller Entoblasts (Fourth Quartette) . .	3
Enteroblasts (Fourth Quartette)	4
Total . . .	11

We will now take up the further history of each of these groups.

1. *The Four Macromeres.*

After the formation of the fourth quartette there is a long interval before the macromeres again divide; during this time the nuclei of these cells become very large and vesicular, and contain one or more large nucleoli, Figs. 52, 86, 87. These cells are composed almost entirely of yolk, and their nuclei and protoplasmic portions lie near the surface just in advance of the edge of ectoblast, and in this position they move around to the ventral pole.

What force is it which carries these nuclei around the egg just in advance of the ectoblast cells? If, as is sometimes assumed, the initial polarity of the egg is due to the fact that the yolk granules have a greater specific gravity than the protoplasm, must it be supposed that the specific gravity of these substances changes in the later stages of cleavage so that the nuclei and protoplasm sink to the lower part of the cell while the yolk rises to the upper part? The progressive movement of the nuclei and surrounding protoplasm over the yolk coincident with the extension of the ectoblast would go against any such conclusion, and would favor the view that this movement

of the protoplasm and nuclei is due to some force other than gravity.

In the process of overgrowth the smaller entoblasts, 4A, 4B, and 4C,[1] are carried around with the ectoblast and mesoblast to the ventral side, and at the same time the whole egg is slightly flattened from above downward, as shown by the enlarged diameter of the egg when seen in polar view, Figs. 49–53, and still better in the actual section shown in Fig. 87. This change of shape is partly due to the fact that these smaller entoblasts, 4A, 4B, and 4C, lie out on the periphery of the egg and thereby enlarge its horizontal diameter, and also to a certain flattening of the macromeres themselves. The changes in the shape of the gastrula are much greater after the next division of the macromeres.

2. *The Fifth Quartette.*

The fourth quartette was separated from the macromeres by laeotropic cleavage. The next division of these cells is more nearly bilateral than spiral, Figs. 54, 57, 60, though it is typical of neither. I shall call the cells thus formed (5A–5D) the *fifth quartette*, in conformity with the designations used for the previous products of the macromeres, though the facts that the division is not a spiral one and that the products are purely entoblastic might, *sensu stricto*, make some other name preferable.

Rarely are more than two of the macromeres seen in division at the same time; in Fig. 54 A and C are dividing first, in other preparations I have found B and C or A and B dividing simultaneously. In D, however, the corresponding cleavage is delayed until long after the division of A, B, and C, Fig. 59, although it was the first cell to divide in the formation of the fourth quartette.

The division of A and B is very nearly bilateral with reference to the second or median cleavage furrow, and the new cells formed, 5A and 5B, lie on each side of that furrow and on the ventral side of the macromeres A and B, Figs. 57, 58.

The cleavage of C and D is less perfectly bilateral than that of A and B, and the new cells, 5C and 5D, are cut off from

[1] Hereafter all entoblast cells are designated by capitals.

the posterior part of the cells C and D, and lie at nearly the same level with the parent cells. This is especially true of the cell 5C which is formed some time before the corresponding cell 5D. The right side of the gastrula after the formation of 5C is accordingly much longer than the left, *i.e.*, the posterior end of the second furrow is carried far to the left by the growth and horizontal division of the cell C (Figs. 58, 59, in which right and left are reversed because seen from the ventral side). With the formation of 5D the left side of the gastrula becomes a little longer and the posterior end of the second furrow is carried back to the right, Fig. 60; but either because the cell 5D is smaller than 5C or because it is given off somewhat more toward the ventral side, the left side of the gastrula remains permanently shorter than the right. *This is the beginning of the final asymmetry of the gasteropod. It is also the beginning of the antero-posterior elongation of the body.* Before the formation of the fifth quartette the transverse diameter of the embryo is as great as the median; after the cells 5C and 5D are formed the median diameter is distinctly longer, Figs. 59, 60, 64, *et seq.* This antero-posterior elongation is due, then, in the first instance to the direction of the cleavage in the posterior macromeres C and D, while the first trace of the final torsion is due to a difference in the time and direction of the cleavage in these two cells.

At the same time that these changes in shape are taking place other changes are going on which lead to the formation of the archenteric cavity, as shown in Figs. 87, 88, 90, which represent actual sections through the egg at this period. Fig. 87 is a transverse section showing two macromeres in the middle and two cells of the fourth quartette, probably 4A and 4C, at the sides; these cells are in process of passing around to the ventral side of the macromeres. Fig. 88 is an oblique vertical section, and shows two cells of the fifth quartette lying on the ventral side of the macromeres and traces of the fourth quartette cells beneath these. Finally, in Fig. 90, which is a transverse section, there is shown in order from above downwards two macromeres, two cells of the fifth quartette, and two cells of the fourth quartette. Between these cells is

the archenteric cavity, the roof of which is formed by the macromeres, and the sides by the cells of the fourth and fifth quartettes. During the process of gastrulation there has been no real invagination of the macromeres, though they have been greatly reduced in size by the formation of the fifth quartette and have also changed their shape, growing broader at the apical pole and narrower at the oral, and finally thinner in the direction of the egg axis, so that they seem to have been invaginated; but this, of course, could not occur, since there is no segmentation cavity.

In later stages a cell, which is almost certainly 5D, moves around to the ventral side of the archenteron, as shown in Fig. 68, where it is seen dividing. By the movements of this cell the archenteric cavity, and in fact all the parts of the embryo posterior to the mouth, are given a distinct torsion. The products of this cell form the floor of the archenteron posterior to the mouth.

3. *The Fourth Quartette.*

Before the fifth quartette is completed by the formation of 5D, the cells 4A, 4B, and 4C divide equally into $4A^1$ and $4A^2$, $4B^1$ and $4B^2$, $4C^1$ and $4C^2$, Figs. 58, 59. The spindles in 4A and 4C are nearly horizontal so that the two cell products lie at approximately the same level; 4B divides in a dexiotropic direction, and the cell products afterwards shift position so that one partly overlies the other, Fig. 60. This cleavage, like that by which the fifth quartette is formed, is neither typically bilateral nor spiral, but intermediate between the two.

The cells thus formed lie close around the blastopore and form the floor of the anterior portion of the archenteron. Ultimately they contribute largely to the formation of the small entoderm cells at the inner end of the stomodaeum.

4. *The Enteroblasts.*

The four enteroblasts can be followed up to Fig. 65 without a break. They lie in the mid line just behind the blastopore and immediately under the ectoderm, and in all cases are closely pressed together in a characteristic grouping. In Fig. 60 one

of them, E^1, is shown dividing, and this is the only case of division among them which I have ever seen; in later stages of other eggs, *e.g.*, Fig. 65, they are still undivided. In Fig. 68 four nuclei are shown beneath the ectoderm and posterior to the cell 5D. They lie at the extreme posterior end of the archenteric cavity, and from their position and grouping I believe they are the nuclei of the enteroblasts.

In Figs. 63 and 65 the anterior enteroblasts, E^1 and E^2, are shown displaced to the right (left in the figure, which is seen from the ventral side). This displacement is probably associated with the rotation of 5D and the laeotropic torsion of all parts posterior to the foot.

5. *Organs formed from the Entomeres.*

(a) *The Archenteron.* — The four macromeres form the roof of the archenteric cavity; the cells of the fifth quartette form its lateral boundaries, inclosing the cavity on all sides save the posterior. Here the archenteric cavity runs backward between the cells 5C and 5D, nearly to the posterior boundary of the egg, Figs. 57, 60, 68. The cells of the fourth quartette come together on the ventral side of the archenteron, forming its floor anteriorly, and ultimately giving rise to some of the many small cells which form that part of the mesenteron, adjoining the stomodaeum, Figs. 90, 93, 95. Thus the whole mesenteron becomes surrounded with yolk cells, all of which have their nuclei next the cavity of the mesenteron.

(b) *The Intestine.* — In the latest stages in which they can be recognized the four enteroblasts form the ventral wall of the posterior prolongation of the mesenteron, Figs. 60–68; and since this prolongation ends near the middle of these four cells, they may also be said to form its posterior wall. This hollow process from the mesenteron is the fundament of the intestine, as further development plainly shows, and it is therefore almost certain that the enteroblasts form a portion of the walls of this organ.

The relation of these cells to the fundament of the intestine is still more plainly shown in actual sections of these stages.

In Fig. 89, which is a median horizontal section through an embryo of the stage shown in surface view in Fig. 74, these cells form the posterior boundary of the mesenteron. In the vertical, longitudinal section shown in Fig. 92 it is also seen that they form the ventral wall of the intestine. The position and histological character of these cells leaves little room for doubt that they have been derived from the enteroblasts.

After the yolk cells have moved over to the ventral side of the mesenteron there is left a polygonal opening between them at the posterior end, and by means of this opening the intestine communicates with the cavity inclosed by the large yolk cells, Fig. 76. By the laeotropic torsion to which the posterior end of the embryo is subjected, this opening is carried farther and farther up on the right side of the embryo, Figs. 68, 76, 80, 81, and at the same time the intestine grows longer, partly by the division of the enteroblasts and partly by the addition of cells which are derived from the yolk cells.

At this stage none of these small intestinal cells contain yolk spherules, and yet they do not stain like the purely protoplasmic cells of the ectoderm or mesoderm, being lighter in color and more transparent. At an earlier stage the enteroblasts contained yolk spherules, Figs. 22–52, but in these later stages they seem to have been dissolved.

The ventral wall of the intestine is from the first composed of these small cells, Figs. 76, 92, while the dorsal wall is formed by the large yolk cells; the intestinal cells, therefore, form a groove, open widely on its dorsal side to the yolk. The two sides of this groove arch over and meet each other first at the distal end of the intestine and then progressively forward from this point; the intestine thus becomes a tube with a distinct lumen, the walls of which are composed entirely of small intestinal cells, Figs. 95, 104, 105. At its anterior end, however, where it opens into the cavity between the yolk cells, the intestine remains a groove, Figs. 93–103.

The posterior opening between the yolk cells, at which point the intestinal cells arise, lies at first on the ventral mid line. In the course of farther development, however, the yolk cells shift their positions, carrying this point of origin of the intes-

tinal cells anteriorly and on to the right side of the embryo, Figs. 76, 80, 81. The blind distal end of the intestine remains for a long time on the ventral mid line, while the main portion of the intestine curves up over the body of the embryo anteriorly and to the right, running just beneath the ectoderm until it ends in the shallow groove of intestinal cells which opens into the cavity between the yolk cells.

A series of horizontal, longitudinal sections through an embryo of the stage shown in Fig. 80 is given in Figs. 102–105. Fig. 102 is taken through the plate of intestinal cells at the upper margin of the yolk-opening, Fig. 80; Fig. 103 is taken through the middle of that opening; while Figs. 104 and 105 are below that opening and show the intestine as a tube; at a little lower level it ends blindly. A thin layer of mesoderm cells surrounds the intestine in the later stages, but is usually separated some distance from its walls, Figs. 80, 81.

In older larvae the torsion goes still farther, the central end of the intestine being carried dorsally and posteriorly, and the distal end being brought up onto the right side of the embryo, where it ultimately opens into the branchial chamber. The anus does not appear until very late, — later than any of the stages figured, — and the proctodaeum is extremely short.

(c) *The Stomach.* — That part of the mesenteron which is bounded by yolk cells we may perhaps, after the example of Bobretzky ('77), call the "stomach," though it gives rise to other structures besides that organ. The yolk cells which bound its cavity are at first large and pyramidal, their bases being outward and their apices extending into the cavity of the mesenteron. The protoplasm of each cell is aggregated at the apex, where also the nucleus is located, while the rest of the cell is densely packed with yolk spheres. At first the ectoderm cells at the inner end of the stomodaeum abut directly against some large yolk cells, Fig. 92, probably the derivatives of 4A, 4B, and 4C; but as development proceeds, the entoderm cells in this region divide repeatedly, growing much smaller, though they are still filled with yolk, while farther back the cells forming the walls of the stomach cavity

remain about the same size as before, Figs. 93, 95, 98. The nuclei of the entoderm cells stain deeply, and they are not round, but angular and irregular, as if they had been pressed out of shape by the yolk spheres which closely surround them. In Figs. 102–104 the entoderm cells at the inner end of the stomodaeum extend around the yolk on the right anterior side nearly to the intestinal cells, while the left and lower part of the yolk is still composed of the large pyramidal yolk cells.

At first the cavity of the stomach is straight. It lies in the median plane of the embryo, and throughout its whole course preserves an antero-posterior direction. When the shell gland and the foot attain to considerable size, the whole posterior part of the embryo is pushed toward the ventral side, and the course of the stomach, while still in the median plane, no longer lies in the line connecting the most anterior and posterior points of the embryo ; its anterior end lies slightly above this line, while its posterior end is much below it, Fig. 92. In more advanced stages the posterior end of the embryo is pushed still farther toward the ventral side, and at the same time is twisted around anteriorly and to the right. The posterior part of the stomach is now bent downward at an angle of nearly 90° with the anterior part, Fig. 93, and at the same time it is turned anteriorly and to the right, and its point of junction with the intestine, *i.e.*, what was primarily its posterior end, is carried up on the right side of the embryo, until, as shown in Figs. 80, 81, it lies far up toward the dorsal surface.

Comparisons.

In its general features the formation of the enteron is essentially the same in all gasteropods so far studied. The intestine, or hinder portion of the alimentary canal, is formed of clear, protoplasmic cells, called the "cylinder cells" by Rabl ('79), the "Darmplatte" by Blochmann ('81), while the stomach is bounded by relatively large cells which contain more or less yolk.

In particular the account of these processes given by Bobretzky ('77) for several prosobranchs is very similar to the account which I have given for Crepidula. However, when one

comes to inquire into the history of individual blastomeres, there is in general the same lack of data which was noticed in the study of the entoblast and mesoblast. In this case, therefore, as in previous instances, I am compelled to limit comparisons almost entirely to the work of Blochmann and Heymons.

I am wholly unable to bring Blochmann's account of the history and fate of the entoderm cells into accord with the work of either Heymons or myself. His figures would indicate that throughout the whole cleavage there is a large segmentation cavity between the four macromeres (see especially his Figs. 40, 49, 51, 58, 60, 64, 67, 71). Three small cells (en_b, en_d, en_x) which apparently correspond to 4A, 4B, and 4C in Crepidula are first shown at the vegetal pole, Figs. 54, 55. *Afterwards these cells pass up through the segmentation cavity,* Figs. 63, 64, until they come to lie on the upper side of the macromeres. In the meantime two new cells are formed (en_a, $en_{a'}$), which have no homologues elsewhere so far as I know, and these cells also pass up through the segmentation cavity to the upper side of the macromeres. These small entoderm cells then divide, forming a cap of small protoplasmic cells which lie above the yolk cells and form the roof of the archenteron. *The latter cavity is in communication with the exterior through the long and narrow segmentation cavity into which the blastopore opens on the ventral side.* These small cells later form the intestine.

This process is so absolutely unique that Blochmann's account may well be doubted. Inasmuch as the macromeres or their derivatives, which are located at the middle of the ventral side, are in many cases invaginated, I once thought it possible that these small cells might correspond to the macromeres, A, B, C, D, while the large yolk cells might represent the cells of the fourth or fifth quartettes ; however, this view cannot be upheld, for these four large yolk cells can be followed right through the development, and are undoubtedly the macromeres A, B, C, D. At present it is altogether useless to attempt a detailed comparison of the entoderm cells of Neritina with those of other gasteropods.

In Umbrella the macromere D is smaller than the other

three, especially after the formation of 4D. The fourth quartette is formed just as in Crepidula, except that the cells 4A, 4B, and 4C are considerably larger than the macromeres, A, B, C, D. There is a fifth quartette in Umbrella, the cells of which are like those of Crepidula in all essential respects, although showing some minor differences. The cell D divides *first*, and C divides soon after. Both of these divisions are equal and bilateral, and the new cells formed (5C, 5D) lie posterior to C and D. Afterward A and B divide bilaterally but unequally, giving off two large cells anteriorly (5A and 5B), which lie in front of A and B and on each side of 4B. Heymons emphasizes the fact that these divisions, unlike the previous divisions of the entomeres, are bilateral, all the spindles being parallel to the median plane.

Afterwards the cells of the fourth quartette 4A and 4C divide in the same direction as in Crepidula; but the anterior product is in each case smaller than the posterior one ; 4B divides much later and in a dorso-ventral direction. It will be remembered that the division of this cell is dexiotropic in Crepidula, and that the products afterwards lie one above the other. In Umbrella this cell (4B) occupies the anterior pointed portion of the embryo.

Those cells of the fourth and fifth quartettes, which are larger than the macromeres in Umbrella, are not carried around to the ventral side as in Crepidula, but, on the other hand, the macromeres move up through the space between these cells. Virtually the same result is thus attained as in Crepidula. In the latter animal the macromeres are the large cells and are relatively fixed in position, while the cells of the fourth and fifth quartettes are smaller and movable; in Umbrella the fourth and fifth quartette cells are the larger ones and are relatively fixed, while the macromeres, which are much smaller, are movable. In both animals the macromeres form the roof of the archenteron, while the cells of the fourth and fifth quartettes form its sides.

Two of the cells of the fifth quartette, 5C and 5D, are quite small and are invaginated with the four macromeres. These six cells Heymons calls the "Primare Darmzellen"; the

remaining entomeres he calls the "Secondare Darmzellen." The intestine is said to be formed from the small cells C″ and D″ (= 5C and 5D). In the history of the entomeres this is really the only point of difference between Umbrella and Crepidula which cannot be satisfactorily explained.

Neither Wilson nor Lillie have observed the division of the entomeres after the formation of the fourth quartette. Mead ('94), however, has observed the formation of the fifth quartette in Amphitrite, for he says, p. 467 : "a4, b4, c4 (= 4A, 4B, 4C) and A, B, C, D form the entoderm, the latter cells each dividing once before the invagination."

VIII. Axial Relations of Egg and Embryo.

1. *The Primary Cleavages.*

In recent years much attention has been paid to the relation of cleavage planes to the future axes of the developing animal. Interest was first awakened in this subject by several observations which tended to show that the first cleavage plane always coincides with the future median plane. Such a relation was found by Agassiz and Whitman ('84) in certain pelagic fish eggs, by Roux ('85) and Pflüger ('85) in the frog, by Van Beneden and Julin ('84) in Clavellina, by Watasé ('90) in Loligo; and the belief was expressed by some authors that the first cleavage plane would be found to coincide with the median plane in all animals with bilateral symmetry. However, further work on this subject has not justified this opinion.

Hatschek ('80) found that in the case of Teredo the first cleavage was transverse to the median plane, and later the same relation was found by Wilson ('90) in Nereis, Conklin ('92) in Crepidula, Heymons ('93) in Umbrella.

In other animals it appeared that the first cleavage lay between the median and transverse planes. Whitman ('78) found in Clepsine that one of the first four macromeres is anterior, another posterior, and that both the first and second cleavage planes cross the median plane of the embryo obliquely. Rabl ('79) established such a relation in Planorbis. Blochmann

('83) found the same thing true of Aplysia, and his figures of the segmenting egg of Neritina ('81) show that here also the first two furrows are oblique to the median plane of the embryo. The same seems to be true of Bobretzky's ('77) figures of Fusus, and Salensky's ('87) figures of Vermetus, though in neither case is this matter mentioned in the text.

In all these gasteropods except Aplysia four nearly equal macromeres are formed ; but in other cases one macromere is often much larger than either of the other three. Among prosobranchs and lamellibranchs this larger macromere is usually in the median line behind, *e.g.*, Nassa, Illyonassa, Urosalpinx, Tritia, Ostrea, Unio, etc., and in all such cases the plane of bilateral symmetry passes through the middle of the larger macromere. Among opisthobranchs and pteropods one or two of the macromeres are frequently smaller than the others, and these lie at the posterior side of the egg, *e.g.*, Aplysia, Umbrella, Cavolina, etc. In such cases the plane of bilateral symmetry may or may not pass through the smallest macromere.

In still other animals it has been found that the first cleavage apparently bears no definite and constant relation to the median plane, *e.g.*, in the toad fish Miss Clapp ('91) has demonstrated that the axis of the embryo may lie in the direction of the first cleavage furrow, or may vary as much as 70° from it; Morgan ('93) asserts that in Ctenolabrus and Serranus "there is no relation whatsoever between the cleavage planes of the egg and the median plane of the adult body"; Jordan ('93) has found in the newt that the median plane may vary more or less from the plane of the first cleavage, and Jordan and Eycleshymer ('94) have found the same thing true of Amblystoma.

Such a series of discordant phenomena might well cause one to regard the axial relations of the first and second cleavages as of no morphological consequence whatsoever. The fact, however, that in many ova there is an absolutely constant relation between the cleavage planes and the axes of the embryo shows that in these cases the position of the cleavage planes and of the resulting blastomeres is not a matter of chance; and this, taken in connection with many other phenomena of similar character,

leads to the view that in those animals with very definite forms of cleavage the position of the furrows and of the cells is causally related to the future axes and organs.

I think it may fairly be doubted whether in any case of spiral cleavage the first furrow is bilateral with regard to the first two blastomeres, even if it is bilateral with regard to the future animal. In Crepidula the first cleavage is radial and the two blastomeres are congruent, not bilateral, antimeres, as is shown by the position of the nuclei and protoplasmic fields subsequent to division, Fig. 6. In all cases the second cleavage is radial, as is shown by the position of the nuclei, and above all by the presence of a polar furrow. Hence, neither of the first two furrows is bilateral with reference to the blastomeres, and therefore neither could be strictly bilateral with reference to the adult. In fact, not a single bilateral cleavage occurs until after the formation of the mesoblast (the cell 4d), and consequently the egg could never, either before or after the formation of 4d, be divided into bilaterally symmetrical halves along the planes of one or any number of cleavages. And yet in many eggs, as has been indicated, the position of the future plane of bilateral symmetry can be determined *approximately* even as early as the first or second cleavage, *e.g.*, Planorbis, Teredo, Nereis, etc. *Such determination, however, is only approximate, since in later stages certain of the micromeres shift across the line of symmetry from one side to the other.*

That the axial relations of the first two cleavages can have no such general significance as was once supposed is beyond question. That they may have, however, very constant and definite relations within or between certain groups of animals, in particular annelids, lamellibranchs, and gasteropods, is the thesis here maintained. At first thought there seem to be many observations opposed even to this limited and modified form of the old doctrine. In fact, among the Mollusca alone three different relations of the first cleavage to the median axis of the embryo are known to exist : (1) it may coincide with that axis, *e.g.*, Loligo ; (2) it may lie at right angles to it, *e.g.*, Teredo, Crepidula, Umbrella; (3) it may cross it at a definite oblique angle, *e.g.*, Planorbis, Neritina, Aplysia. The

first is found only in a highly modified form of cleavage ; the two latter are characteristic, so far as is certainly known, of all gasteropods, lamellibranchs, and annelids, and if the thesis mentioned above is to be maintained, these differences must be explained. This, I believe, can be done, and in the following manner :

In Crepidula and all other animals with spiral cleavage in which there is a marked difference in size between the macromeres and micromeres, the first and second cleavage planes represent in the main the furrows between four entoderm cells. *These cells may have different axial relations in different cases, but so far as known the ectoderm and mesoderm cells to which they give rise always have the same axial relations.*

Thus no exception is known either among mollusks or annelids to the rule that the second and fourth quartettes lie in the future median and transverse planes, and that the first, third, and fifth quartettes lie midway between these planes. *The axial differences, therefore, of the first two cleavage planes, which have been mentioned, are differences merely in the axial relations of the four primary entoderm cells, and do not affect the axial relations of the other cells of the ovum, which are always the same among annelids and mollusks.*[1]

That this is really true is further shown by the fact that in all cases in which the first and second furrows coincide with the transverse and median planes respectively, the whole of the ectoblast and mesoblast rotates around the egg axis until the second and fourth quartettes lie in the median and transverse planes; whereas in all forms in which one macromere is anterior, one posterior, one right, and one left, there is no such rotation (*cf.* cross in Neritina and Crepidula). In all known cases of spiral cleavage, excepting a few sinistral gasteropods, the micromeres are separated from the macromeres in the same

[1] Lillie ('95) has expressed somewhat similar views on this subject. He mentions three cases, *viz.*, Clepsine, Planorbis, and Neritina, in which the macromeres are anterior, posterior, right and left respectively; but he discusses only the first of these cases, and concludes that "at present we are unable to explain why, when widely separated forms agree, nearly related species should show reversed relations." The other two cases, *viz.*, Planorbis and Neritina, show clearly that these reversed relations concern only the four macromeres.

direction and occupy the same relative positions : *e.g.*, the cross is formed over the middle of each macromere, but since in all cases it occupies the same position relative to the future axes, while the macromeres may occupy different positions, it follows that it must rotate in some cases and not in others.[1]

Coenogenetically the macromeres have been much modified, witness their differences in size; and the difference in position is probably another such modification. Whether in later stages even these differences disappear is not certainly known, but even if four cells do occupy different positions in different species, it is a small matter compared with the fact that hundreds of cells forming the most important organs of the body occupy the same positions. These facts show that both phylogenetically and ontogenetically the position of the ectomeres and mesomeres is more fundamental than the position of the entomeres. A proper comparison, therefore, between the axes of different animals in these early stages is to be found in the position of the ectoblast and mesoblast rather than in that of the entoblast. Such a comparison would show, I think, that in all annelids and mollusks (excepting, of course, the cephalopods) the median and transverse axes lie in the same groups of ectomeres and mesomeres, and it might possibly unify the varying results thus far obtained in the study of the cleavage in fishes and amphibians.

2. *Establishment of the Larval Axes.*

Hatschek ('88) has expressed in the terms *Protaxonia* and *Heteraxonia* the fact that in one great group of animals, the Coelenterata, the primary axis of the ovum becomes the chief axis of the larva and of the adult, while in all animals with bilateral symmetry these two axes are not the same. In the typical trochophore larva, *e.g.*, that of Polygordius, this change of axis is accomplished by the forward movement of the blasto-

[1] In accordance with the description of these rotations given in the earlier part of this paper, I here regard the macromeres as fixed and the micromeres as rotating. In view of the conclusion here reached it would be much better to reverse the process and consider the micromeres fixed in position and the macromeres changeable.

pore (vegetative pole) to one side of the larva, which then becomes the ventral face. This movement is usually accompanied by the closure of the blastopore from behind forward. In some trochophores, however, especially the larvae of the Mollusca, in which there is a large accumulation of yolk at the vegetative pole, this pole may remain fixed in position while the animal pole is shifted forward. The end result is the same in the one case as in the other. In still other cases the movement takes place at both poles; in fact in all cases it is probable that both poles shift position a little.

The result of this shifting is that the egg axis is bent, the angle growing greater and greater throughout development. This bending of the egg axis caused by the shifting of the poles may all be referred ultimately to the greater and more rapid development of the posterior side of the egg, and this greater growth may be located more specifically in the group of cells derived from the first and second somotablasts (2d and 4d), and is associated with the formation of the trunk. The continued elongation of the trunk in annelids is accompanied by the development of metameric segmentation ; in mollusks it leads to a ventral curvature of the trunk associated with the formation of the shell on the postero-dorsal surface, and in gasteropods this curvature of the longitudinal axis is still further complicated by the laeotropic torsion of the trunk region posterior to the foot.

In Crepidula the conditions are precisely like those described above for ova with a large amount of yolk. The chief axis of the ovum becomes the principal axis of the gastrula ; this axis is at first a straight line, but in later stages its upper end is carried forward through an angle of about 90°. In fact, this bending of the embryonic axis is so great and sharp that it is doubtful whether one should not regard that axis as altogether destroyed. The longitudinal or antero-posterior axis runs from the apical cells in front to the anal cells behind, and is perpendicular to the earlier position of the embryonic axis.

The blastopore closes in the middle of the ventral side, Figs. 65, 66, and its posterior lip does not appear to grow more rapidly than the anterior or lateral lips. This might

seem to indicate that the posterior growth of the embryo was not greater than the anterior or lateral growth, but such is not the case, as is strikingly shown by an examination of the aboral side of the embryo, Figs. 64, 74. It is here seen that while the greater growth of ectoblast at the posterior side of the egg has not changed the position of the blastopore, it has shoved the whole of the ectoblast on the aboral surface forward through an angle of about 90°. In this shifting of the ectoblast the entoblast cells take no part, as is clearly shown by the position of the four macromeres and the polar furrow both before and after this movement of the micromeres, Figs. 64, 74. At this stage the polar furrow marks the middle of the dorsal area, the blastopore lies at the middle of the ventral area, and the apical cells which form the centre of the cross lie on the anterior side of the egg about 90° from either of these points. This forward shifting of the apical pole is due to two causes : first, to the enormous enlargement of the cells of the posterior cell plate (Figs. 74, 77), and second, to the rapid multiplication of cells in the region of the shell gland.

In later stages by the enlargement of the shell gland and the multiplication of cells just ventral to it (the growing-point) the ectoblast of the ventral surface is shifted forward, so that the mouth is carried anteriorly, Fig. 76, until it comes to lie near the apex, as shown in Fig. 78. At the same time the embryo increases greatly in length, chiefly by growth at the posterior end, and marks of the final asymmetry appear. The posterior end of the embryo is marked during all this time by the anal cells and by the distal end of the intestine, Figs. 76–78; the antero-posterior axis is at first a straight line connecting the apical and the anal cells, Fig. 77.

This change of axis is undoubtedly the same phenomenon which has been described by Fol ('75 and '76) in the case of pteropods and heteropods, by Blochmann ('81) in Neritina, by Heymons ('93) in Umbrella, and by Lillie ('95) in Unio. The last-named observer has given such an excellent summary of all these cases except that of Neritina that I need not discuss them here.

In one point Blochmann's account of this shifting of the axes in Neritina differs from my observations on Crepidula.

Blochmann finds the cause of this forward movement of the ectoblast in the formation of an ectodermic invagination at the apex. Apart from the doubtful character of this invagination, to which I have already referred, there is such a mass of evidence in favor of the view that the forward movement is due to the greater growth at the posterior pole that I think the cause assigned by Blochmann may fairly be called in question.

In some gasteropods, in which there is a small quantity of yolk, the change of axis occurs in the method typical for the trochophore, *viz.*, the apical pole does not change position, but the oral pole is shoved forward on the ventral side, probably by the development of a structure homologous with the ventral plate of the annelids. Such a process has been described by Patten ('86) in the case of Patella.

These shiftings of the embryonic axes are apparently characteristic of all Mollusca, and they present no essential difference from the methods which Alex. Goette ('82) has shown are characteristic also of turbellarians, nemertians, and annelids.

The question of the origin of bilateral from radial forms, and the consequent establishment of the antero-posterior axis among all bilateralia is one which has received much attention from some of the most eminent men of science.

Lankester ('77) long ago suggested that the antero-posterior axis of bilateral animals corresponds to the chief axis of coelenterates, the oral pole of the radiate forming the anterior pole of the bilateral animal, and the aboral part of the coelenterate lengthening to form the trunk of the bilateral animal.

Balfour, on the other hand, held that "the conversion of such a radiate form into the bilateral took place not by the elongation of the aboral surface and the formation of an anus there, but by the unequal elongation of the oral face, . . . while the aboral surface became the dorsal surface." This conclusion was elaborated by Balfour, and after him by Sedgwick. Both supposed the central nervous system of arthropods, annelids, mollusks, and chordates to be derived from a circumoral nerve ring.

After proposing this theory, Balfour says (*Comp. Embryology*, vol. II, p. 379): "The position of the flagellum in Pilidium

and of the supraoesophageal ganglion in Mitraria suggest a different view of the origin of the supraoesophageal ganglion. . . . The position of the ganglion in Mitraria corresponds closely with that of the auditory organ in Ctenophora ; and it is not impossible that the two structures may have had a common origin. If this view is correct, we may suppose that the apex of the aboral lobe has become the centre of the preoral field of the Pilidium and trochosphere larval forms."

This view has been most carefully and elaborately presented by Alex. Goette in his classical work *Entwicklungsgeschichte der Würmer*. He divides bilateral animals into two groups : (1) the pleurogastric, in which the chief axis of the egg becomes the chief axis of the larva or adult, *e.g.*, Sagitta and the echinoderms, and (2) the hypogastric, in which group one of the "cross axes" of the egg becomes the chief axis of the larva or adult, *e.g.*, worms, mollusks, arthropods. Goette has striven to show that in all bilateralia the animal pole (*Scheitelpol*) corresponds to the future cephalic pole (*Hirnfeld*), and so far as his hypogastric forms are concerned his views on this subject have found repeated and abundant confirmation in the more minute and exact studies which have been made during the last few years on the development of annelids and mollusks.

3. *Beginnings of Final Asymmetry.*

The development of the characteristic asymmetry of the gasteropod belongs to a later period than is treated of in this paper. However, the beginnings of that asymmetry are clearly marked during the embryonic period, and may be briefly touched upon here.

The first evidence of asymmetry and the first trace of anteroposterior elongation appear at the same time and are apparently due to the same cause, *viz.*, the formation of the fifth quartette. The posterior members of this quartette, 5C and 5D, are cut off from the posterior side of the macromeres C and D (Figs. 57–60), thereby increasing the length of the median axis, and, because 5C is formed earlier and lies nearer the dorsal side than 5D, a certain torsion of the posterior end of the embryo follows. This torsion increases until 5D lies nearly on the mid-ventral

line, while 5C lies on the dorsal side, Fig. 68. At the same time all structures on the ventral side are carried to the right, *e.g.*, the fundament of the intestine, while structures on the dorsal side are displaced to the left, *e.g.*, the fundament of the shell gland, Fig. 74. The ectoderm and mesoderm seem to follow the entoderm cells in this torsion, as if they were being passively shifted by the movements of the latter.

In later stages, with the evagination of the shell gland, Fig. 78, the posterior end, morphologically, is shoved farther and farther toward the ventral side. By the latter movement the distal end of the intestine is carried forward on the ventral side, by the laeotropic torsion it is moved up on to the right side of the embryo, Figs. 80 and 81, until the alimentary canal crosses itself like a figure 8 open at the top. These two motions combined bring about the complicated form of asymmetry characteristic of the adult.

With the evagination of the shell gland, the yolk cells protrude like an immense hernia through the lips of the gland, being covered, however, by an exceedingly thin layer of large ectoderm cells; this portion of the embryo becomes the visceral mass (*Eingeweidesack*). The point at which the shell gland was first formed marks the summit of the spire of the adult shell, and the lips of the invagination become the mantle edge, as is well known.

It is abundantly evident from this account that the asymmetry of the adult Crepidula is not produced by the asymmetrical development of the shell gland, as is usually maintained for gasteropods in general. In fact the initial asymmetry of the shell gland is produced by the torsion of the posterior end of the embryo. In Crepidula the first recognizable cause of the torsion lies in the asymmetry of the cells 5C and 5D. That this has any profound phylogenetic significance, however, seems to me rather doubtful. The yolk cells, because of their great size, exercise an undue amount of influence upon the shape of the entire embryo. It seems to me that phylogenetically neither the yolk cells nor the shell gland were the source of the torsion; they merely took part in a general twisting of the entire posterior end of the embryo.

Crampton's ('94) observation that in a sinistral gasteropod, Physa, there is a reversal of the usual directions of spiral cleavage, is particularly interesting in this connection. If the initial asymmetry is caused in Physa, as in Crepidula, by the asymmetry of the cells 5C and 5D, then it is easy to see how this reversal of the cleavage stands in a causal relation to the reversed asymmetry of the adult. Both Crampton and Kofoid ('94) call attention to the fact that in Rabl's ('79) figures of the embryology of Planorbis there is a reversal of the usual direction of cleavage, and also that in Haddon's ('82) figures of Janthina a similar reversal is indicated. In Planorbis the asymmetry of the adult is reversed, though this does not seem to be the case in Janthina (*cf.* Kofoid ('95), p. 69).

If it should prove on further investigation that reversed cleavage always leads to reversed asymmetry of the adult, there would be good reason for believing that Crepidula exhibits the usual and perhaps the ancestral method in the establishment of the asymmetry of the gasteropods.

D. GENERAL CONSIDERATIONS.

1. *The Forms of Cleavage.*

Several different kinds of cleavage are commonly recognized: (1) with reference to its extent, cleavage is total (holoblastic) or partial (meroblastic); (2) regarding the relative size of the cell products it is equal or unequal; (3) in the distribution of yolk it is telolecithal or centrolecithal; (4) with reference to the constancy of form, it is regular or irregular; (5) with reference to symmetry it is radial or bilateral. To these five classes I think a sixth should be added, at least for the present, *viz.*, one with reference to the destiny of the cleavage cells and axes, and for which I propose the names determinate and indeterminate. It may be that future work will show that this distinction is not necessary, but in the present state of knowledge such a distinction exists and it is very useful to have a name for it. Attention is directed in this place only to the two

last-named classes, *viz.*, that having reference to the symmetry of cleavage and that having reference to its prospective value in the developing organism.

(a) *The Radial Type.*

(1) Orthoradial Cleavage. — The purely radial form of cleavage, in which a series of "meridional" furrows alternate with a series of "equatorial" ones, and in which the cleavage cells as well as furrows form zones and meridians which are perpendicular the one to the other, has long been regarded as the typical form of all total cleavage. Thus, in the earlier works on the cleavage in the frog, Amphioxus, mollusks, echinoderms, in fact most animals, it is usually stated that there is a regular alternation of meridional and equatorial furrows, and that the number of cells is doubled at every stage; accordingly it was once supposed that the entire number of cells could be accurately determined by getting the approximate number of nuclei present on one side of the egg. And even to this day there are those who lightly speak of 64-, 128-, 256-, and 512-cell stages in a way that causes one who has ever attempted a detailed study of cleavage, especially in these later stages, to stand aghast. Rauber ('82) has rendered great service by pointing out the fact that in the frog's egg all such representations are pure diagrams which have no counterpart in nature. Nevertheless these ideas of cleavage have found secure and undisputed lodgment in many of the text-books, and so the error is propagated from year to year, and from generation to generation. All such ideas of a purely radial, or orthoradial,[1] type of cleavage with the cells regularly increasing in geometrical ratio are misleading, if not absolutely erroneous. Instead of being the usual form of cleavage, this orthoradial type is exceedingly rare and is never found beyond

[1] Wilson designates as a "purely or truly radial" form of cleavage that in which "there are two systems of cleavage planes, of which one set are meridional and radially symmetrical to the egg axis, while the other set intersect the meridians at right angles." I propose for this form of cleavage the term *Orthoradial*, since the so-called "spiral" form of cleavage is just as truly and purely radial as the other.

the first few divisions. Wilson ('92 and '93) cites Amphioxus, Echinus, Synapta, Antedon, and Sycandra as typical examples of the orthoradial type of cleavage. In his work on Amphioxus, however, he says that the radial form is present in only about three-quarters of all the eggs of that animal, and that even in these the "cross furrow" may or may not be present. The presence of a polar or cross furrow is in itself sufficient evidence that the cleavage is not strictly orthoradial. Among the echinoderms I have observed that polar furrows are usually present in the eggs of Asterias and Arbacea, and I believe that some of the cases in which orthoradial cleavage is figured may be attributed to the influence of the usual teachings upon this subject. But granting that there are some eggs, as appears to be true, in which the cleavage is orthoradial up to the 8- or 16-cell stage, there is certainly no egg which preserves the orthoradial condition through any considerable part of the cleavage. This form of cleavage is not only very rare, but when found it is exceedingly evanescent and very soon gives way to spiral cleavages. Driesch ('92) has well said: "Das so oft schematisch gezeichnete Vierzellenstadium mit zwei sich in zwei Puncten schneidenden Meridianen kann man wohl getrost aus der Reihe des Existirenden streichen; vgl. hierzu die genauen Furchungsstudien von Chabry und Rauber. Das Princip der kleinsten Flächen, dessen nothwendiger Ausdruck (Plateau, Lamarle) es ist, dass stets drei Flächen in einer Kante, vier Kanten in einem Punkt zusammenstossen, scheint gerade in der Ontogenie der Thiere besonders deutlich zu Tage zu treten."

(2) Spiral Cleavage. — The word "spiral" has long been used (Selenka '81, Lang '84) to describe that form of cleavage in which there is an actual or virtual rotation of the blastomeres upon each other. Wilson ('92), however, first proposed the recognition of a *spiral type* of cleavage. "The spiral type," he says, "arises from the radial through a twisting of the radii, as it were, the blastomeres being displaced or rotated, with respect to the egg axis, either to the right, following the hands of a watch (right-handed spiral), or in the reverse direction (left-handed spiral), as the case may be.

. . . The term 'spiral' refers to the fact that the curved radii, if prolonged, would form a spiral about the egg axis." Lillie ('95), commenting on this statement, says: "In the ontogeny there is no twisting of the radii, but merely an inclination of the axis of the dividing cell from the vertical. It seems to me, therefore, that this form of cleavage would be more correctly termed *oblique*." Kofoid ('95) has also criticised the term spiral cleavage as ambiguous and misleading, and suggests as a substitute *alternating cleavage*. It seems to me, however, that the name used by Wilson is better than either of these later suggestions. Lillie's only objection to the term, apparently, is the fact that there is no twisting of the radii; in Crepidula, however, there is in the early cleavages just such a twisting of the radii as Wilson mentions, dependent upon the actual rotation of the blastomeres after they have been formed.[1] It is true that in many animals with this type of cleavage an actual rotation of the blastomeres and the consequent twisting of the radii do not occur, but in all such cases there is at least a *virtual* rotation. But the principal reason for preferring the term *spiral* to the word *oblique* is that it has long been used (Selenka, Lang, Wilson, Heymons) in one form or another to designate that kind of oblique cleavage in which the divisions are in the same direction in each quadrant, so that it has come to have in this connection a distinctly technical meaning, whereas, as commonly used, *oblique* cleavages may be in different directions in different quadrants, *i.e.*, they may be bilateral or radial or neither. The suggestion made by Kofoid merely emphasizes the alternating character of the successive cleavages, and this might be more satisfactorily accomplished by the use of the term *alternating spirals*, since alternation is as characteristic of orthoradial as of spiral cleavages. There are cases, as we shall see a little later, in which the cleavage is oblique in the same direction in each quadrant (*i.e.*, it is spiral), *but in which it does not alternate with the preceding*

[1] Almost all who have written on the cleavage of gasteropods (Fol, Rabl, Blochmann, Heymons) describe an *actual rotation* of the cells. In Crepidula this rotation is especially well shown in the formation of the first and second quartettes and in the subsequent division of the first; it is not marked in the formation of the third quartette, but is very pronounced in the separation of the fourth.

cleavage. It will not do, therefore, to lay too great emphasis on the alternation of cleavages. It must be confessed that the expression *spiral cleavage* is open to objection, since in many cases no real spiral is formed either by the spindles or the cell walls. Still, if taken apart from its colloquial meaning, the word *spiral* clearly and specifically designates a particular kind of cleavage which needs a distinctive and technical name, and it may be doubted whether any other name would not be open to as serious criticism.

On the other hand, Wilson's exposition of the spiral type of cleavage is in certain places open to objection. He says ('92, p. 378): "The cell divisions . . . show a peculiar modification of radial symmetry, which is best characterized as spiral in character, and which cannot be reduced to the bilateral type." The suggestion, here contained, that there is a third kind of symmetry, *viz.*, *spiral symmetry*, is still further borne out by the three coördinate types of cleavage which he establishes, *viz.*, the radial, the spiral, and the bilateral. That such a classification, either of symmetry or of cleavage, is unjustifiable is shown, I think, by the fact that so far from being "a peculiar modification of radial symmetry," the *spiral symmetry*, thus suggested, is one of the most common forms of radial symmetry, and likewise the "spiral type of cleavage" is by all odds the most common representative of radial cleavage. Spiral cleavages, therefore, belong entirely to the radial type, and should not be classified as coördinate with either the radial or bilateral types.

I shall limit the use of the term *spiral* to those cases in which cleavage occurs in the same direction in each quadrant, *i.e.*, it is always a purely radial cleavage. If this radial character is changed even in one out of four quadrants, it would then be better to use the term *oblique*. Oblique cleavages then might be or might not be bilateral, but they would not be radial. As we shall see later, oblique cleavages, using the term in this special sense, are transitional between spiral and bilateral cleavages.

One of the most constant and characteristic features of all radial cleavage is the alternation of direction in successive

divisions. This alternation is essentially the same in both orthoradial and spiral cleavages; in the former case, the axes of the nuclear spindles are alternately meridional and equatorial, in the latter they lie between these positions, being alternately oblique to the right and to the left.

It is a most remarkable fact that in all known cases of spiral cleavage, with the exception of a few sinistral gasteropods, the direction of the spirals is invariably the same. The full significance of this fact can only be grasped when one realizes that spiral cleavages are found in animals so far apart as turbellarians, annelids, lamellibranchs, and gasteropods.

Selenka ('81) first called attention to the spiral character of the *third* cleavage by which the first quartette of ectomeres is formed. He also observed that in the formation of the second quartette the spiral was in the opposite direction. Lang ('84) carried the spiral cleavages back to the *second* division of the egg, which he characterized as a "left-wound spiral." As a result of this spiral division, he showed that two of the macromeres lie at a higher level than the other two, and consequently two polar furrows are formed (see p. 52). These polar furrows always bear a fixed relation to the first two cleavages, because the second cleavage is constantly laeotropic.

Other investigators have recognized the spiral character of the second cleavage in many other animals, but, so far as I know, no one has suspected that the *first* cleavage also is a spiral one. This, however, is the case in Crepidula, for immediately after the first cleavage is completed, it can be seen that the first division was a *dexiotropic* one.[1] Likewise *in all animals in which the second cleavage is constantly laeotropic, it is probable that the first is virtually dexiotropic. The spiral cleavages, therefore, probably begin with the first division of the egg, and in almost every case in a dexiotropic direction, the second division is laeotropic, the third dexiotropic, the fourth laeotropic, etc.* In Crepidula these alternating spirals proceed without a break, except slight differences in the time of division in the different quadrants, from the 1- to the 44-cell

[1] See p. 42.

stage, and even after this they continue in a majority of the cells as long as the cleavage can be followed.

Kofoid ('95) has collated from the most important literature on spiral cleavage the facts as to alternation, and has presented them in a series of excellently constructed tables. In conformity with the methods used by him, there are given in the following table (pp. 180, 181) the facts as to the alternation of successive cleavages in Crepidula.

This table shows one very clear case of the reversal of an entire spiral cleavage, *viz.*, the division of the basal cells of the cross $1a^{1.2}$, etc., Gen. VII. Two other cases, not so clearly marked because the spindles are nearly meridional or equatorial, are found in the division of the cells $2a^{2.1}$, etc., Gen. VIII, and the descendents of these cells, $2a^{2.1.1}$, etc., Gen. IX.

There are many cases in which reversals are seen in one quadrant, while the usual direction is preserved in the other three. Thus, every division of the third quartette (with the possible exception of $3a^{1.2}$, etc.) shows reversal in at least one quadrant, and the same is true of certain cells of the first and second quartettes. These reversals, however, unlike those which occur in all four quadrants, have reference to the appearance of bilateral symmetry.

In Neritina there is a total reversal of the cleavage in the basal cells of the cross $1a^{1.2}$, etc., just as in Crepidula, whereas in Umbrella the cleavage of these cells follows the usual rule. I have elsewhere (p. 95) pointed out the fact that upon this reversal the continued existence of the cross as a recognizable structure in Neritina and Crepidula depends. In Neritina the second division of the third quartette ($3a^{1}$, etc.) is indicated in Blochmann's figures (see Diagram 12, *b*), and this shows reversals in quadrants B and D, so that the divisions are purely bilateral; in Crepidula there is a reversal in quadrant D only, so that the cleavage is bilateral in the posterior quadrants, but not in the anterior ones. Umbrella shows almost exactly the same reversals in the history of the third quartette as are exhibited by Crepidula. All three of these gasteropods show a slightly greater tendency to reversals in quadrants B and D than in quadrants A and C.

TABLE GIVING DIRECTIONS OF CLEAVAGES IN CREPIDULA.

(*Cases of reversal are in italics.*)

GENERATION.	NUMBER OF CELLS.	DESIGNATION OF CELLS.	DIRECTION OF DIVISION.	FIGURES.
I	1	Ovum		
II	2	AB, CD	Right	2–6
III	4	A, B, C, D	Left	7–11
IV	8	A, etc. $<^{\text{A, etc.}}_{\text{1a, etc.}}$	Right	12 & 13
V	12	A, etc. $<^{\text{A}}_{\text{2a}}$	Left	14 & 15
	16	1a, etc. $<^{\text{1a}^{\text{I}}}_{\text{1a}^{2}}$	Left	16
VI	20	A $<^{\text{A}}_{\text{3a}}$	Right	17
	24	2a $<^{\text{2a}^{\text{I}}}_{\text{2a}^{2}}$	Right	18 & 19
	29	$1a^{I}$ $<^{1a^{I.I}}_{1a^{I.2}}$	Right	22
	—	$1a^{2}$ $<^{1a^{2.I}}_{1a^{2.2}}$	Bilateral (divides in quadrants A and B only)	50
VII	25 & 52	A $<^{\text{A}}_{\text{4a}}$	Left	21 & 33
	34	3a $<^{3a^{\text{I}}}_{3a^{2}}$	Left in A, B, C; *Right in D* (not constant)	25–28
	38	$2a^{I}$ $<^{2a^{I.I}}_{2a^{I.2}}$	Left	26–28
	42	$2a^{2}$ $<^{2a^{2.I}}_{2a^{2.2}}$	Left in A, B, C; *Right in D* (not constant)	26–28
	88	$1a^{I.I}$ $<^{1a^{I.I.I}}_{1a^{I.I.2}}$	Left	44–47
	47 & 77	$1a^{I.2}$ $<^{1a^{I.2.I}}_{1a^{I.2.2}}$	*Right* (*reversed spiral*)	31 & 42
VIII	—	A $<^{\text{A}}_{\text{5a}}$	Bilateral	54 & 57–60
	—	4a $<^{4a^{\text{I}}}_{4a^{2}}$	Right (?)	58 & 59

Generation.	Number of Cells.	Designation of Cells.	Direction of Division.	Figures.
VIII	64 & 68	$3a^{1} < 3a^{1.1}, 3a^{1.2}$	Right in A, B, C; *Left in D* (almost equatorial)	36 & 38
	68 & 77	$3a^{2} < 3a^{2.1}, 3a^{2.2}$	*Left* in A, B, C; Right in D (almost equatorial)	42 & 43
	77 & 88	$2a^{1.1} < 2a^{1.1.1}, 2a^{1.1.2}$	Right in A, B, D; *Left in C* (not constant)	44–47
	58 & 64	$2a^{1.2} < 2a^{1.2.1}, 2a^{1.2.2}$	Right	35 & 38
	58 & 64	$2a^{2.1} < 2a^{2.1.1}, 2a^{2.1.2}$	*Left* (almost meridional)	35 & 38
	—	$1a^{1.1.1} < 1a^{1.1.1.1}, 1a^{1.1.1.2}$	Bilateral	53
	—	$1a^{1.1.2} < 1a^{1.1.2.1}, 1a^{1.1.2.2}$	Bilateral, *Left in B and D*	51
	111	$1a^{1.2.1} < 1a^{1.2.1.1}, 1a^{1.2.1.2}$	Right in A, B, C; no division in D (almost meridional)	46 & 47
	77	$1a^{1.2.2} < 1a^{1.2.2.1}, 1a^{1.2.2.2}$	Right in A, B, C; no division in D (almost meridional)	42
IX	111	$3a^{1.1} < 3a^{1.1.1}, 3a^{1.1.2}$	*Right in A and B;* no division in C and D	46 & 47
	111	$3a^{1.2} < 3a^{1.2.1}, 3a^{1.2.2}$	Left	44–47
	—	$2a^{1.1.1} < 2a^{1.1.1.1}, 2a^{1.1.1.2}$	Bilateral in A and C; *Right in D*	49 & 56
	—	$2a^{1.1.2} < 2a^{1.1.2.1}, 2a^{1.1.2.2}$	Bilateral in A and C; *Right in D*	49 & 56
	111	$2a^{1.2.1} < 2a^{1.2.1.1}, 2a^{1.2.1.2}$	Left in A, B, C	46 & 47
	88	$2a^{1.2.2} < 2a^{1.2.2.1}, 2a^{1.2.2.2}$	Left in A, B, C	44–47
	111	$2a^{2.1.1} < 2a^{2.1.1.1}, 2a^{2.1.1.2}$	*Right* (almost equatorial)	46 & 47
	111	$2a^{2.1.2} < 2a^{2.1.2.1}, 2a^{2.1.2.2}$	Left (almost equatorial)	46 & 47
	—	$1a^{1.2.1.1} < 1a^{1.2.1.1.1}, 1a^{1.2.1.1.2}$	Bilateral (*Right in A and C;* no division in D)	53
	—	$1a^{1.2.1.2} < 1a^{1.2.1.2.1}, 1a^{1.2.1.2.2}$	Bilateral (Left in A, B, C; no division in D)	53
	—	$1a^{1.2.2.1} < 1a^{1.2.2.1.1}, 1a^{1.2.2.1.2}$	Bilateral (*Right in A, B, C;* no division in D)	49
	—	$1a^{1.2.2.2} < 1a^{1.2.2.2.1}, 1a^{1.2.2.2.2}$	Bilateral (Left in A, B, C; no division in D)	49

In the following table the reversals in Crepidula are classified according to quadrants :

TABLE OF REVERSALS OF CLEAVAGE IN CREPIDULA.

In all four quadrants.	Quadrant A.	Quadrant B.	Quadrant C.	Quadrant D.
$1a^{1.2}$, etc. $2a^{2.1}$, etc. (?) $2a^{2.1.1}$, etc. (?)	$3a^{2}$ (?) $1a^{2}$ $3a^{1.1}$ (?) $1a^{1.2.1.1}$ $1a^{1.2.2.1}$	5b $3b^{2}$ (?) $1b^{1.1.2}$ $3b^{1.1}$ $1b^{1.2.2.1}$	$3c^{2}$ (?) $2c^{1.1}$ $1c^{1.2.1.1}$ $1c^{1.2.2.1}$	3d $2d^{2}$ 5d $3d^{1}$ $1d^{1.1.2}$ $2d^{1.1.1}$ $2d^{1.1.2}$
Total cases . . 3 Doubtful . . . 2	5 2	5 1	4 1	7 0

Dropping all doubtful cases of reversal, in which the spindles are nearly meridional or equatorial in position, there remain three cases in quadrant A, three in C, four in B, and seven in D.

To this table of reversals there should be appended a statement that *eight* divisions which occur in the anterior quadrants, A and B, are not represented in quadrant D, and likewise *two* are not represented in quadrant C. All of these omitted cleavages except two ($3c^{1.1}$ and $3d^{1.1}$) are connected with the peculiar history of the posterior turret cells and the posterior arm of the cross.

In a few cases which are classified here as reversals the nuclear spindle does not indicate that the cleavage is to be reversed, and even the daughter nuclei may occupy the same relative positions as in the quadrants in which there is no reversal, while at the same time the lobing of the cytoplasm and the subsequent rotation show that the cleavage is reversed ; the first division of 3d (Figs. 25, 26, 29) is a case in point. In such cases the conditions which influence the direction of the cleavage are not manifested until after the nuclear division is completed, whereas they are usually shown in the direction of the nuclear spindles and in the earliest stages of cleavage.

(b) *The Bilateral Type.*

In purely bilateral cleavage, such as is found among the ascidians and cephalopods, the very first division is bilaterally symmetrical. In other cases bilaterality may not be clearly marked at the beginning of the development, but still may appear at a very early stage.

Wilson ('93) has found in Amphioxus that the cleavage of normal eggs may be bilateral or spiral or radial in the earliest stages, although there is shown "a distinct tendency toward bilaterality in almost all forms of the cleavage."

In annelids, gasteropods, and lamellibranchs, on the other hand, the cleavage is typically spiral until about the time of the formation of the mesoblast (4d). In Nereis, according to Wilson, this spiral cleavage suddenly gives place to the bilateral. "The most striking feature in the cleavage," says Wilson, "and the one on which the entire discussion may be made to turn, is the sudden appearance of bilateral symmetry in the cleavage; . . . *the bilaterality does not appear at the beginning of development.* It appears only at a comparatively late stage, and by a change so abrupt and striking as to possess an absolutely dramatic interest." "The bilateral asymmetry of the early stages depends mainly upon the fact that the substance of the somatoblasts (*i.e.*, the mesoblast and the material of the ventral plate) is stored in the left posterior macromere. Bilateral symmetry is established upon the reduction of this macromere (D) to the size of its fellow (C) by the separation of the somatoblasts and their transportation to the median line. Immediately upon this event follows the appearance of bilateral cleavages throughout the embryo, except in the cells which give rise to the prototroch, a purely larval organ."

Among the mollusks conditions are very different, bilateral cleavages appearing very slowly, and creeping, as it were, from cell to cell and from quadrant to quadrant. In Crepidula they first appear at the 34-cell stage and by a slight shifting in position of a single cell, $3d^2$, immediately after its formation; so slight is this movement that it is doubtful whether it ought to be considered as indicative of bilateral cleavage. The

next bilateral cleavage occurs at the 42-cell stage, and it also consists of a slight change in the direction of division of the single cell $2d^2$. The first bilateral division in the mesoblast occurs at the 44-cell stage, and all subsequent divisions in this layer are bilateral. From this time on bilateral cleavages increase in number, but up to the stage with 111 cells perfect spiral cleavages are present, and in the very latest stages to which any group of cells could be traced spiral cleavages were found in some of the cells (usually three) of each group. (See table of Directions of Cleavage.)

That the reason assigned by Wilson for the "bilateral asymmetry" of the early stages is not applicable here is shown by the fact that in many of the gasteropods the left posterior macromere is not appreciably larger than the right and in some (*e.g.*, Umbrella) it is smaller, and also by the fact that the mesoblast (4d) is only one member of a quartette which is separated in a left spiral from the macromeres, each of the other members being quite as large as, or even larger than, the cell 4d. The following conclusions may be drawn concerning the origin of bilateral cleavages among the gasteropods :

(1) Bilateral cleavages first appear on the posterior side of the egg.

(2) They are generally due to a reversal of the direction of cleavage of one out of four cells, this reversal being most frequent in quadrant D.

(3) Certain time differences appear between the divisions on the anterior and posterior sides of the egg, the divisions on the posterior side being much slower in the first quartette, but ultimately much more rapid in the second and third.

(4) Another factor in the establishment of bilaterality, and the one which gives meaning to the three preceding ones, is the teloblastic growth of all the layers at the posterior end of the embryo and the formation in this region of the larger part of the adult body.

(5) The primitive radial symmetry is preserved in the anterior quadrants long after it has disappeared in the posterior ones, *e.g.*, the arms of the cross, the origin of larval mesoblast in quadrants A, B, and C, etc.

The conclusion, therefore, is unmistakable that bilaterality first appears in processes which lead to the formation of the trunk and the elongation of the future animal, while the primitive radial symmetry of the anterior quadrants, which is so long preserved, is correlated with the fact that these quadrants give rise largely to larval organs, most of which bear traces of radial symmetry.

(c) *Significance of the Forms of Cleavage.*

The cause of alternating cleavages in general has been very fully discussed by various writers, particularly by Sachs (*Physiology of Plants*, ch. XXVII) and by Hertwig (*Die Zelle und die Gewebe*, ch. VI). The latter author has presented, in the form of two laws, the principles upon which alternations in division are based. These laws are : (1) the nuclear spindle lies in the direction of greatest elongation of the protoplasm; (2) the division walls intersect the spindles and the previous division planes at right angles. It is probable that these principles are true in general, but they meet with many exceptions in the development of most animals. The chief objection to these laws is that they assume that protoplasm is an inert substance which behaves during and after division like so much clay. On the other hand, nothing is more certain than that protoplasm has intrinsic powers, which are, at least occasionally, capable of setting aside these mechanical principles : *e.g.*, it is able to change its shape so that it may elongate twice or a dozen times in the same direction, as is seen in most cases of teloblastic growth; or the axis of the nuclear spindle may lie in the shortest diameter of the protoplasm, and the division take place apparently in the direction of the greatest pressure (cf. McMurrich, '95[1] and '95[2]); or the division wall may intersect the spindle obliquely (as I have observed in several cases in Crepidula) ; or successive division walls may intersect each other at any angle from 0° to 90°. The setting aside of these as well as many other mechanical principles on the part of living matter is due to the fact that protoplasm is not soapsuds or oil emulsion, but something vastly more complex than either; and it gives evidence that in cleavage, as in

the entire development, intrinsic factors of development are more important than extrinsic ones.

Reversal of cleavage may be due, apparently, to either of the following causes: (1) it may be produced by external mechanical disturbances which compel a second division in the same direction, or (2) it may be caused by the precocious appearance of certain organs or planes of symmetry. In most cases of normal cleavage I believe it can be shown that the first cause is dependent upon the second, and that the ultimate cause of reversals is therefore an intrinsic one.

All that one can affirm concerning the so-called "law of alternating cleavage" is that in early stages successive cleavages tend to alternate in direction if uninfluenced by processes of differentiation. This law of alternation is less manifest in the later than in the early stages of development, and even in the early stages it may be violated as soon as definite cell groups, *e.g.*, the cross, begin to appear.

Apart from the general phenomenon of alternations in cleavage we may now consider the significance of the peculiar features of radial and bilateral cleavages.

(1) Significance of Orthoradial Cleavages. — The most remarkable thing concerning orthoradial cleavage is that it does not conform to the principle of minimal contact surfaces. So far as I can recall, all eggs which are said to exhibit this form of cleavage, *e.g.*, Amphioxus, echinoderms, Scyandra, do not exhibit a compact form, but consist of a number of blastomeres loosely piled together, and generally with a large segmentation cavity between them. During the early stages of cleavage these blastomeres are individualized to such an extent that they are globular and are not closely pressed against their neighbors. There are therefore no rotations of the blastomeres, and consequently no polar furrows or pressure surfaces. The compactness of the egg is sacrificed to the independence of the segment spheres. It may be worth while to remark in passing that this independence in form is generally associated with a great amount of independence in function, as experiment has demonstrated in the case of echinoderms and Amphioxus. Sooner or later these independent blastomeres

lose their rounded outlines ; they are pressed more and more closely together, rotations occur, and consequently pressure surfaces are developed, and we have the principle of surface tension and, perhaps, of mutual attraction between the cells (*Cytotropismus*, Roux '94) asserting itself in the appearance of *spiral cleavages*.

(2) Significance of Spiral Cleavages. — So far as the mere rotation of blastomeres and the consequent formation of polar furrows and pressure surfaces is concerned, I quite agree with Wilson that "the spiral type owes its peculiarities entirely to mechanical conditions, the blastomeres assuming the position of greatest economy of space, precisely like soap bubbles or other elastic bodies." This form of cleavage alone fulfills the conditions of minimal contact surfaces, and considered from the purely physical standpoint, it is a wonder that it should ever fail to occur. Spiral cleavages, then, in general, are certainly due to the general physical phenomenon of surface tension ; but the fact that they occur in definite directions is just as certainly due to something else. The absolute constancy of direction in certain cases of spiral cleavage is a thing which no merely extrinsic factors can possibly account for. The alternate directions of the spirals is but an expression of alternation in general, and each successive cleavage finds the sufficient cause of its direction in the direction of the preceding one until we reach the first cleavage. Why is the first division dexiotropic rather than laeotropic ? I cannot at present answer this question, but it is obvious that the cause of this constancy of direction must be intrinsic rather than extrinsic, and that it must be sought for, not in the mechanical conditions of surface tension, but rather in the structure of the unsegmented egg itself.

The direction of the spirals has presumably a profound influence upon the entire development. Associated with it is the formation of the mesoblast and the greater part of the adult body from the left posterior macromere in cases where the spirals are not reversed, whereas it is the *right* posterior macromere which gives rise to these structures in cases of reversed cleavage, as Crampton ('94) has shown in the case of

Physa. And the fact that the asymmetry of the adult body is reversed in those gasteropods (Physa, Planorbis) which show reversed cleavage makes it probable that the direction of the spirals influences not only the cleavage stages but also the entire development.

The general significance of radial cleavages, both orthoradial and spiral, may be considered here, since it has much to do with the interpretation of cleavage in general. The mere alternation of divisions explains many fundamental features of radial cleavage, but it by no means touches upon its most interesting and remarkable characters. These characters are particularly well shown, not only in the radial symmetry manifested in the *direction* of division, but especially in the *size* and *shape* of the blastomeres. Unequal cleavages in themselves, as I have argued elsewhere, must signify more than extrinsic forces; they can be explained only by assuming certain intrinsic causes, and when we have these unequal cleavages minutely repeated in the different quadrants, even though the mechanical environment in those quadrants may be different, we have to reckon with causes which are still more complex and obscure.

As striking illustrations of this radial symmetry in the position, size, and shape of cells may be mentioned the following: the formation of four macromeres, frequently equal in size; the formation of at least three, usually four, quartettes of micromeres; the radial symmetry manifested in the history of each quartette, unless modified by the early appearance of definitive structures; the radial symmetry of embryonic or larval structures, such as the trochoblasts, the apical cells, the terminal and basal cells of the cross, the reversed cleavage in each of the latter, and the long-continued resemblances between the right, the left, and the anterior arms of the cross; the origin of the mesoblast from the second quartette in quadrants A, B, and C, and from the fourth quartette in quadrant D.

In several cases these radial structures seem to belong to the same category as the radial structures of the trochophore larva,

and I believe that they are to be explained as a foreshadowing of larval characters, just as bilateral cleavages are usually attributed to a precocious development of adult characters.

Wilson ('93) emphasizes the fact that bilaterality in cleavage is an inherited character. This is undoubtedly true, but it is also just as true that radiality in cleavage is an inherited character. It is possible to conceive of a radiality which would be due merely to extrinsic forces and stresses, but this is not the radiality of cleavage; for so far as now known the latter is characterized by a definiteness in the directions of division and in the size and form of the resulting cells, which such extrinsic forces are wholly unable to explain.

It seems to me highly probable that all forms of cleavage are truly inherited, just as certainly as the size and shape and character of the egg or spermatozoön are inherited. The loose character of the aggregate of blastomeres in Amphioxus, the compact form of cleavage with its definite spirals in the annelid or mollusk, the bilateral arrangement of the blastomeres in the ascidian, all are ultimately due to the same thing, *viz., the structure of the germinal protoplasm.* These peculiarities could not be produced by extrinsic forces, they must come from within; and, if I understand the word at all, this is just what distinguishes *heredity.* On the other hand, certain minor features in all these forms of cleavage are due to extrinsic factors, and consequently the forms of cleavage, like all other forms of the organism, are the resultants of the intrinsic and of the extrinsic factors of development.[1]

(3) Significance of Bilateral Cleavages. — In the case of bilateral cleavages the law of alternations or rectangular intersections is violated more or less from the beginning; and likewise the principle of minimal contact surfaces is more or

[1] In a review of Wilson's work, Driesch ('95) criticises this very point in a way with which I thoroughly agree. He finds the cause of all different kinds of cleavage in the structure of the protoplasm, and hence concludes that one is as truly inherited as the other. It seems to me that this conclusion differs radically from some of his earlier views concerning cleavage; indeed, I am unable to harmonize it with other expressions in this same paper, *e.g.*, he says that there can be no phylogenetic significance in the close resemblance between the cleavage in annelids and gasteropods *because it has been mechanically produced* (see p. 195).

less completely set aside. It seems quite certain, therefore, that the cause of the bilateral form of cleavage is an intrinsic, not an extrinsic, one. If it be true that there are cases of bilateral cleavage which have no reference to the bilaterality of the adult, as Miss Clapp's ('91) observations on the toadfish and Morgan's ('93) on certain teleosts indicate, it can only be explained, so far as I can see, by supposing that the same causes which operate to produce bilaterality in the adult may operate independently on the cleavage stages, producing bilateral symmetry which has no connection with that of the adult. This probability seems to me so remote that I think it more likely, considering the extensive shiftings and rotations of blastomeres which have been observed in some animals, that the bilaterality of cleavage is only an early appearance of the final bilaterality with which it is directly continuous, *though perhaps only after extensive shiftings of cell groups, or even of entire layers, have occurred.* The further possibility remains that in some cases apparently bilateral cleavages are not really bilateral, but are radial, as is the case with the first cleavage in Crepidula.

(d) *Determinate and Indeterminate Cleavage.*

In only a comparatively small number of animals, so far, has the history of individual blastomeres been traced through the development to the organs which they ultimately form. In a few cases, however, among such widely separated groups as Annelida, Gasteropoda, Lamellibranchiata, Arthropoda, and Tunicata, this has been done in the case of a few cells, and with the constant result that, under normal conditions, definite cells in any given animal invariably give rise to definite structures in the embryo or the adult. Such cells are not only identical in origin and destiny but also in shape, size, and developmental history. Such definiteness in the origin, form, and history of blastomeres leads irresistibly to the view that the history of each cell in such ova is, under normal circumstances, predetermined and always in the same way and to the same end. For all such kinds of cleavage I propose the name *determinate cleavage.*

On the other hand, in most Echinodermata, Coelenterata, and Vertebrata, no such definiteness in the history of the blastomeres is known to exist. Of course the possibility remains that in most, if not all, of these cases the cleavage is of just as determinate a character as in the first class mentioned, and that the denial of a definite prospective value to each blastomere must rest upon the curious basis that no one has followed a single blastomere through the development. I confess that to me this possibility seems extremely probable.

Under present circumstances, however, it would be unjustifiable to classify all cleavage as equally determinate in character, merely on the grounds of analogy with such cases as the annelids and mollusks. There is some evidence that the extent of predetermination differs in different cases (see Wilson, '93 and '94); I propose, therefore, to classify all cases in which predetermination is not known to exist as *indeterminate cleavage*. Such a classification is in many respects an unsatisfactory one, and it can only be regarded as having a temporary value, but it will serve to emphasize a distinction which in our present state of knowledge we must recognize as existing.

Most of the earlier experimental work in embryology was done upon forms in which the cleavage is not known to be determinate in character, and many general conclusions were drawn which are not applicable to determinate cleavage. For example, some of Driesch's conclusions have been too sweeping; no one who has ever studied such determinate forms of cleavage as are exhibited by the annelids and the mollusks could for a moment admit the truth of his earlier conclusion ('93): "By segmentation perfectly homogeneous parts are formed capable of any fate." There is every ocular evidence that in the cases referred to, the parts separated by cleavage are not perfectly homogeneous, and under such circumstances to assert that they are would be the climax of self-stultification.

There is, I think, a fallacy in Hertwig's much-quoted dictum ('92): "In consequence of the continuity of development, every older cell group must arise from a younger cell group, and so finally definite parts of the body from definite segment cells." A true conclusion would be this: "And so finally definite parts

of the body from any cell you please." The fact that definite parts of the body come from *definite cleavage cells* means more than the mere continuity of development, and in this very fact the whole question at issue between determinism and indeterminism is contained.

Later work, particularly that of Wilson ('92) on Nereis, Driesch and Morgan ('95) on Ctenophore eggs, and Crampton ('96) on Illyonassa, have led to important modifications of these extreme views. Driesch now sees in cleavage something more than the mere sundering of perfectly homogeneous materials. He grants, what one cannot fail to observe in many cases of determinate cleavage, the existence of cytoplasmic differentiations in certain cleavage cells, and even in some cases in the unsegmented egg (v. Driesch and Morgan ('95), p. 221). He still maintains, however, that the possibility of predicting the prospective significance of single cells is simply a result of the continuity of development as Hertwig's dictum asserts.

2. *Cell and Regional Homologies.*

In looking for the earliest appearing homologies between different animals, embryologists have generally been content to stop with the germ layers. One of the first and most successful attempts to go back of germ layers was made by Professor Whitman ('78) in his classical work on the embryology of Clepsine. Since then, under the stimulus of his work and suggestion, there have appeared, chiefly from the Marine Biological Laboratory at Wood's Holl, a remarkable series of contributions on this subject of the earliest homologies (cf. Wilson '92 and '93, Lillie '93 and '95, Mead '94, Conklin '92). Owing in large part to the work of this school, there are now sufficient data at hand for making an extensive comparison of every step in the development of a number of annelids, lamellibranchs, and gasteropods.

(a) *Cell Homologies among Annelids and Mollusks.*

Until recently there has been an evident tendency to regard cleavage in different families and orders as exhibiting only general and not detailed resemblances. Thus Bobretzky ('77)

believed that mollusks had only the gastrula form in common with other animals. Wolfson ('80) and Fol ('76), who maintained that there were agreements between the early cleavage stages in gasteropods and lamellibranchs, were opposed by Rabl ('79), Hatschek ('80), and Blochmann ('81), who held that there were no detailed resemblances. Blochmann concluded that the cleavage in Chiton does not belong to the gasteropod type; and although he pointed out several resemblances between the cleavage of gasteropods and of turbellarians, no one supposed that outside the molluscan phylum any exact or long-continued resemblances to molluscan cleavage would be found. I recall with what astonishment Professor Wilson and the writer found, only a few years ago, that the cleavage of Nereis and Crepidula was so wonderfully similar in many respects. Wilson ('92) called attention to many of these resemblances, though at that time I think he did not suspect that they were as numerous nor as precise as they have since been found to be. Lillie's ('95) work added some very important points of resemblance between the cleavage stages of the annelids and the mollusks, and in this work I have been able to add still others.

Wilson ('92) emphasizes the following important resemblances between the early cleavage stages of the annelid, the polyclade, and the gasteropod: (1) the *number and direction of the cleavages* is the same in all three up to the 28-cell stage; (2) in general the cells formed are *similar in position and size, viz.*, there are four macromeres, three quartettes of micromeres, and the first quartette is surrounded by a belt composed of the second and third quartettes. The first quartette undergoes three spiral divisions in alternate directions, and the second quartette divides once. Here the resemblance with the polyclade ceases, though the annelid and gasteropod go one step further in these likenesses, *viz.* (3), the *three quartettes of micromeres are ectomeres* in the annelid and gasteropod, and (4) in both these groups *the mesoblast is formed from the cell 4d*, which gives rise to paired mesoblastic bands.

Beyond this point Wilson believed that the annelid diverged from the gasteropod. He supposed that the "cross" in the two was wholly different both in origin, position, and destiny,

and that the velum had a wholly different origin from the annelidan prototroch.

Lillie ('95) has extended all the above-mentioned resemblances between annelids and gasteropods to the lamellibranchs, and in addition has discovered the following: (5) the *first somatoblast* (*2d*), which gives rise to the ectoderm of the trunk, has exactly the same origin and position and a similar history in the annelid and lamellibranch; (6) it gives rise to a *growing-point* and a *ventral plate* in all respects essentially like those of the annelids. Lillie shows good reason for believing that in other mollusks the posterior growing-point is derived from these cells.

To this list of resemblances between the annelid and the mollusk, which I can confirm in the case of the gasteropod, I have been able to add the following: (7) the *rosette series* of the gasteropod is exactly like the *cross* of the annelid in origin, position, and probably in destiny. The *intermediate girdle cells* of the annelid are like the *cross* of the gasteropod in origin, position, and destiny (at least in part). The differences, therefore, between the annelidan and molluscan cross which Wilson emphasizes are not real ones; (8) the *trochoblasts* of the annelids are precisely similar in origin and destiny (at least in part) to the *turret cells* of the gasteropods. In some annelids (Amphitrite, Clymenella), the prototroch is completed by cells of the same origin as in Crepidula and Neritina. The differences which Wilson points out between these two structures do not therefore exist. In both annelids and mollusks the prototroch lies at the boundary between the first quartette on one side, and the second and third on the other. In both there is found a preoral, an adoral, and a post-oral band of cilia; (9) in the gasteropod the apical cells give rise to an *apical sense organ* such as is found in many annelid trochophores; (10) the *supra-oesophageal ganglia and commissure* apparently arise from the same group of cells in annelids and gasteropods; (11) the *fourth quartette* in annelids and gasteropods contains mesoblast in quadrant D, but is purely entoblastic in quadrants A, B, and C; (12) a *fifth quartette* is formed in gasteropods and some annelids (Amphitrite, etc.), and consists of entoblast only; (13) in the gasteropod *larval mesoblast* arises from the same group

of ectoblast cells as in Unio, differing, however, in this regard that it is found in quadrants A, B, and C, whereas in Unio it is found in quadrant A only; (14) to this list of accurate resemblances in the cleavage cells may be added the fact that *among annelids and mollusks the axial relations of all the blastomeres (except possibly the four macromeres) are the same.*

What a wonderful parallel is this between animals so unlike in their end stages! How can such resemblances be explained? Are they merely the result of such mechanical principles as surface tension, alternation of cleavage, etc., or do they have some common cause in the fundamental structure of the protoplasm itself? Driesch answers ('92): "The striking similarity between the types of cleavage of polyclades, gasteropods, and annelids does not appear startling; it is easy to understand this, since cleavage is of no systematic worth."[1] To this, I think, it need only be said in reply that if these minute and long-continued resemblances are of no systematic worth, and are merely the result of extrinsic causes, as is implied, then there are no resemblances between either embryos or adults that may not be so explained. And conversely, these resemblances in cleavage, however they have been produced, stand upon the same basis with adult homologies.

Within the group of the annelids Wilson ('92) says that "adult homologies are represented by accurate cell homologies in the cleavage stages." But in his general interpretation of

[1] The entire passage (Driesch ('92), p. 41) reads as follows: "Es sind also gewisse äussere Umstände, welche die Furchung beherschen, in Form *empirischer Gesetze* ganz oder nahzu bekannt. Wir können daraus immerhin Manches lernen, so wird uns die auffallende Ähnlichkeit, welche die Furchungstypen von Polycladen (Selenka, Lang), Gasteropoden (Rabl, Blochmann, Fol, etc.), und Anneliden (Wilson) darbieten, nicht so sehr frappiren; *wir haben eine leises Verständniss dafür gewonnen, wesshalb Furchungsbilder nicht systematisch verwerthbar sind.*"

In similar vein he affirms elsewhere ('95, p. 416): "Wenn auch nicht durchaus, so sind also doch in sehr wesentlichem Masse der Furchungsbilder mechanisch verständlich, wofür auch die Thatsache spricht, dass bei Nereis, bei Polyclade, und bei Gasteropoden nahezu identisch gestaltet sind; das spricht zugleich gegen ihren Werth für phylogenetische Abtheilungen." It should be noted that if cleavage is inherited, as Driesch affirms elsewhere in this same paper, and if certain forms of cleavage are characteristic of species, genera, families, and orders, as is unquestioned, cleavage does have phylogenetic significance, whether that significance can be extended to widely different types, such as the polyclades and the gasteropods, or not.

cleavage he points out some fundamental differences between these early stages in the annelids, gasteropods, and polyclades, and concludes (p. 455): "Blastomeres having precisely the same mode of origin and precisely the same spatial relations to the rest of the embryo are by no means necessarily equivalent either physiologically or morphologically, and the early cleavage stages in themselves have little morphological value."

Lillie ('95) has taken much more positive ground for the homology of blastomeres among annelids and mollusks, and he was justified in so doing because of the truly wonderful resemblances which he was able to demonstrate between the lamellibranch and the annelid.

I have attempted to show that the differences of cleavage between the annelids and the gasteropods, upon which Wilson lays emphasis, are only apparent and not real, and that therefore we cannot deny the general homology of blastomeres among annelids, gasteropods, and lamellibranchs.

Concerning the polyclade cleavage I can offer nothing new. The differences here are very great, perhaps irreconcilable, and certainly this is true of other types of cleavage, such as the bilateral, the centrolecithal, and the meroblastic. But to affirm the homology of blastomeres within certain groups is not to assert that they are everywhere homologous, nor that they are completely homologous. The mesoderm of the adult mollusk differs very considerably from that of the annelid, the trunk region in the two groups is widely different, and we need not expect to find the protoblasts of these structures completely homologous.

The fact is there are no *perfect* homologies between adult annelids and mollusks, and therefore we need not expect to find *perfect* homologies between their larvae, germ layers, or cleavage stages; but, since final homologies are invariably based upon earlier ones, we should expect to find that blastomeres in general show resemblances and differences corresponding to the resemblances and differences of the end stages, and this is just what we find in the cases mentioned.

An incidental result of these observations is to bring the annelids and mollusks more closely together than has heretofore

been done. It has been generally conceded that the trochophore larva which appears in the development of both of these groups is evidence of their former connection, but the resemblances mentioned above show that in the prelarval stages, and also in the metamorphosis following the trochophore stage, there are many resemblances between the two groups, particularly in the history of the somatoblasts, the formation of the trunk, and the establishment of bilateral symmetry.

On the other hand, the embryological history only serves to widen the gap between the cephalopods and other mollusks, for in the early development there is apparently nothing in common between the two.

The application of the word *homology* to pregastrular stages may deserve a short explanation and justification. This term as employed by Owen was used to denote morphological correspondence in the relative structure, position, and connection of adult parts; but since this morphological correspondence is characteristic of the parts of embryos as well as of adults, it is evident that to rigidly limit the word *homology* to adult characters would be to draw a wholly artificial and useless distinction between adult and embryonic structures. Accordingly, we find that the word has been very generally used to denote morphological correspondence of embryonic, as well as of adult, parts, and this correspondence was found in earlier and earlier stages of the ontogeny, until Huxley finally homologized the germinal layers of higher metazoa with the cell layers of adult coelenterates.

The chief objections which have been raised in recent years against the general homology of the germ layers arise from the fact that the layers in themselves have been regarded as organs which might be compared as if they were adult parts. They were estimated by what they were rather than by what they might become, and consequently false ideas often obtained as to what they really were; for the real structure of embryonic parts can usually be determined only by observing the entire history of those parts. I presume no one supposes that we can directly recognize and compare the fundamental structure of eggs, blastomeres, or layers; at present the only satisfactory way of

determining whether they have the same structure is to observe what they develop into. If certain embryonic parts always give rise to certain definitive structures, the conclusion is warranted that these parts themselves must be alike in structure. The homology of germinal layers, therefore, must have reference to prospective resemblances, and accordingly the test of all such homologies must be the history and destiny of those layers (cf. Wilson, 95).

If, however, prospective resemblances form a basis for homology, there is no reason for stopping with germ layers in seeking to find the earliest homologies. In those cases in which an entire layer can be reduced to a single cell, how is it possible on morphological grounds to affirm homology of the layer but to deny it to the cell? Is it not evident that an altogether unnatural distinction is made when an imaginary line is drawn between blastomeres and layers, on the one side of which homologies may be predicated and on the other not?

If organs which are homologous among annelids and mollusks, such as the prototroch, the apical sense organ, the stomodaeum, and the ventral plate, can be traced back in their development to certain individual cells of similar origin, position, size, and history, are not these cells truly homologous? If not, where in this developmental process shall we say that homologies begin?

I believe there is no escape from the conclusion that the protoblasts of homologous organs are as certainly homologous as are the organs to which they give rise, that the protoblasts of homologous layers are as surely homologous as are those layers, and that the protoblasts of definite regions are as much homologous as are those regions. We therefore reach the conclusion that, in related organisms with determinate cleavage, homologies may be predicated of single cells, whether they be protoblasts of the nervous system, the excretory system, or the locomotor apparatus; of the ectoderm, the mesoderm, or the endoderm; of the right or left, the anterior or posterior portions of the body.

It does not matter, so far as the fundamental idea of cell homology is concerned, how such homology may have arisen. The definite character of the cleavage of Nereis is ascribed by

Wilson ('92) to *precocious segregation*. Lillie ('95) maintains that it is *parallel precocious segregation* that conditions cell homologies. What the cause of this parallel precocious segregation, or of precocity in general, may be is a matter of much doubt.

The term *precocious segregation* was first introduced by Lankester ('77), to indicate the fact that the segregation of parts or layers might be "pushed back into the egg." From the expressions which are frequently used in this connection, such as the "pushing of characters back into the egg," "the reflection of adult characters back upon the egg," etc., it seems that the process is commonly considered a direct rather than an indirect one; or, in other words, that adult characters appear earlier in successive generations, owing to the influence of the body plasm upon the germ plasm. This distinct form of Lamarckism is apparently held by embryologists who repudiate that doctrine in any other form; it is, however, as can be seen by a moment's thought, the very centre and stronghold of the Lamarckian doctrine. On the other hand, it is possible to explain precocity in development by assuming that eggs show multifarious variations, and that natural selection has picked out such as are most beneficial to the species. In fact, there is no doubt that eggs show repetition and variation phenomena as truly as do adult organisms, and they would therefore afford a field for the action of natural selection.

No satisfactory or conclusive evidence as to the cause of precocity can at present be furnished, but the following observations may help to an ultimate solution of this problem:

(1) Adult characters have influenced embryonic characters, and especially cleavage stages, more than the latter have influenced the structure of the adult. This principle finds very many illustrations, among which may be mentioned the following: great individual variations of cleavage produce slight, if any, variations in the adult, as is shown normally in the case of Renilla and Amphioxus (Wilson) and experimentally in the case of many different animals (Driesch, Hertwig, Wilson, Morgan, etc.). Many determinate characters of cleavage, which can have little or no significance for the egg itself, are yet of

importance in building the adult; among these may be mentioned the early appearance of bilateral symmetry; the appearance of bilateral symmetry in diverse directions in the different layers always associated with the future rotation of some of the layers in a definite direction ; the segregation of materials for certain organs, layers, and regions of the body into definite cells ; the distribution of yolk to the various blastomeres, being found in some cases in many cells, in others being largely confined to a single cell ; all these and a hundred other determinate characters have only a prospective value and must have been produced, either directly or indirectly, by the influence of the later upon the earlier stages.

(2) Precocious differentiation, while indicating a shortening of the *process* of development, does not indicate a shortening in its *duration.* Many animals of high organization run through their development in a very short time and yet show no traces of precocity, while many lower animals, although showing a high degree of precocity, yet develop very slowly; *e.g.*, the chick develops in twenty-one days, Crepidula reaches its larval stage only at the end of four weeks, and yet in the former case no precocity is apparent in the early stages, whereas it appears at the very beginning of development in the latter. Even within the limits of a single group the rate of development varies greatly, though apparently the precocity does not ; *e.g.*, the relatively rapid development of pteropods as compared with prosobranchs.

Numberless instances might be given to show that the rapidity of development does not depend upon the amount of yolk contained in the egg, as the text-books always have it, nor upon the temperature at which normal development occurs, but rather upon the individual peculiarities of the protoplasm itself (cf. Kofoid '95 and Castle '96). The shortening of the time of development, therefore, is not in any way correlated with precocious differentiation, and hence it is unwarrantable to assume that the latter has been produced by natural selection, owing to the beneficial effects of the former.

(3) Precocity does not insure the development of a larger number of individuals, nor does an egg which manifests pre-

cocity produce more perfectly and more surely the adult organism. The percentage of abnormal forms among animals which show no precocity is no greater than among those with pronounced precocity. (See abnormalities of development among gasteropods, p. 30.)

(4) It seems probable that by a shortening of the process of development there would be a distinct saving of energy; for if we regard only the energy expended in nuclear and cell division, it is possible to see that in an organ which reaches functional activity after a dozen divisions less energy has been expended than in one which reaches this stage only after one hundred divisions. *To this saving of energy precocious segregation may in general be due.*

The "reflection" of similar larval or adult characters would produce similar effects upon different eggs, and consequently *the similarity of the prelarval stages of annelids and mollusks may be held to be due to the similarity of their larvae;* but there is no reason for supposing that this parallel precocity has been *independently* acquired by annelids and mollusks, since it may well have been produced before the phylogenetic separation of those groups.

(b) *Regional Homologies.*

It is certain that a considerable number of accurate cell homologies are found among annelids, lamellibranchs, and gasteropods, but such homologies cannot at present be claimed for all the cells of the cleaving eggs of these animals; and between these and other groups which manifest determinate cleavage, *e.g.*, Turbellaria and Ctenophora, it is probable that no such accurate cell homologies exist. As has been argued elsewhere, one ought not to expect more complete homologies among blastomeres than among organs. In most cases, however, which have been carefully investigated, homologous organs come from the same *regions* of the cleaving egg. This is a fact of the most general application and of the greatest importance. Apical sense organs, cerebral ganglia, and the ectoderm in general come from the animal pole, the entoderm comes from the vegetal pole, while the mesoderm usually comes from the region be-

tween the two. Polar differentiation, however produced, seems to be essentially the same in all cases.[1] In very many bilateral animals the animal pole forms the cephalic end of the antero-posterior axis, though it frequently undergoes great shiftings to reach that point. So far as known, the trunk region of annelids and mollusks always comes from the same region of the embryo. The prototroch always comes from the region between the first and second quartettes of ectomeres. There is good reason to believe that in all cases each quartette of ectomeres occupies homologous regions in the adult. In all these cases there are fundamental homologies of regions, though homologous parts may not always be limited by homologous cell walls, as Whitman ('94) has argued. The fact, however, that so many accurate cell homologies exist among several different groups seems to me to indicate that the formation of cells has a more important rôle in development than Whitman assigns to it (cf. Wilson, '94).

CONCLUSIONS.

In general, the forms of cleavage are the result of three distinct classes of factors, which may vary in importance in different animals. (1) The first and simplest of all are the *mechanical* conditions, such as surface tension, alternation of cleavage, and the like. These conditions are always present and are generally, though not invariably, fulfilled, the result being that certain fundamental features of all cleavage may be referred to such factors.

(2) The fact that cleavage is an inherited character and that definite forms of cleavage and accurate cell homologies are characteristic of several great groups of animals gives it a certain *phylogenetic* value, for however they may have been produced, inherited structural likenesses which run through closely related species, genera, orders, and types must be considered to have a phylogenetic value. The fact that such likenesses are real homologies, as has been argued elsewhere, is evidence upon this point.

[1] One remarkable exception to this statement is known. Castle ('96) has found that the polar bodies are formed at the vegetal (entoderm) pole in Ciona, and the same is probably true of other ascidians.

(3) The principal significance of any determinate form of cleavage is *prospective* rather than *retrospective;* almost every peculiar feature of determinate cleavage can be referred directly to its usefulness in building the body of the future animal.

The cause of this determinate character of cleavage is not to be found primarily in known mechanical conditions nor the extrinsic factors of development, but rather in intrinsic structures, conditions, and forces. In the category of phenomena, which at present can be explained, so far as I can see, only by referring them to such intrinsic causes, may be mentioned the following: (1) the dexiotropic direction of the first cleavage with the consequent alternation in direction of every succeeding cleavage up to an advanced stage; (2) the reversal of the usual direction of cleavage in the formation of certain definite structures, or in the establishment of bilateral symmetry; (3) the establishment of bilateral symmetry in diverse directions in the different germinal layers, and the subsequent coincidence of these different planes of symmetry in a common plane; (4) the general phenomenon of the unequal division of apparently homogeneous cells; (5) the rapid growth and slow division of certain cells (*e.g.*, the trochoblasts) and the slow growth and rapid division of other cells (*e.g.*, the apicals); (6) the segregation of the ectoblast into three quartettes of cells, and the formation of the mesoblast in the fourth quartette.

These are but a few specific cases and many others might be mentioned; in fact, the most important phenomena of development must be included in this category, — among them the proper collocation of parts and coördination of results, all cases of precocity and determinism, and, in fact, the ultimate cause of all specific and generic characters, some of which are frequently manifested at every stage from the beginning to the end of development. Each and all of these phenomena can at present be attributed only to intrinsic causes, since known mechanical conditions are wholly unable to explain them.

The ground here taken is not one of opposition to the possibility of a mechanical explanation of vital phenomena; such

an explanation Biology, as a causal science, is bound to seek after and expect. It is only against that narrow and near-sighted view which mistakes aims for achievements and which would explain all the mysteries of development by such known forces and conditions as gravity, surface tension, cohesion, viscosity, and the like that exception is here taken. It is safe to assert that, before any such explanation can be given, our conception of mechanics in general must be greatly enlarged. The mechanical explanation of vital phenomena is a great task, and one not to be accomplished in a year or a century. We ought not to deceive ourselves by supposing that we have already reached, or are indeed near, such an explanation.

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SUPPLEMENTARY NOTE.

Renewed study of Figs. 56, 62, 65–73 renders it probable that the interpretation of certain cells in those figures, as shown in the plates and explained in the text, is erroneous. I have already called attention to the fact that, at the time of the formation of the fifth quartette (Fig. 54), the entire egg becomes irregular and many landmarks disappear. Owing to this fact it is extremely difficult to follow the lineage through this period. I have all along been aware of certain discrepancies in the interpretation of some of the figures mentioned but supposed that this might be due to variations in the time of division and in the size of resulting cells; *e.g.*, in Fig. 53 there are, in the anterior arm of the cross, eight cells arranged in two rows, four in a row. In Fig. 56 there are apparently only six cells, three in each row. In Fig. 62 the number is indefinite, though only the basals could be plainly recognized.

I am now inclined to the view that the large paired cells marked V′ in Figs. 62, 69, 70, and 71, and $1b^{1.2.2.2.2.2}$ in Figs. 65, 66, 67, 68, 72, and 73 are the same and that they are identical with the *middle cells* of the anterior arm ($1b^{1.2.2.1.2}$ and $1b^{1.2.2.2.2}$, Fig, 50). Accordingly in all these cases the cells lying apical to these middle cells are the *inner* and *outer basals*, Fig. 53. Throughout all these figures the inner basals remain well marked, the outer ones, however, undergo division, forming in Figs. 69, 70, and 71 two large and two small cells ($1b^{1.2.2.1.2}$ and $2b^{1.1.1}$). The latter, which should be labelled $1b^{1.2.2.2.2}$, are probably thrown away, the former remain as the narrow cells ($1b^{1.2.2.2.1}$, Figs. 65, 66, 67, 68) just apical to the middle cells while on the apical side of these are the inner basals. In Fig. 62 the four small cells lying between the inner basals ($1b^{1.2.1.1.1}$, etc.) and the middles (V′) are probably the derivatives of the outer basals, no one of which has yet been thrown away. *According to this interpretation, the first velar row runs through the tip cells of the anterior arm just as it does through the tip cells of the right and left arms, while a portion of the outer basals of the anterior arm, and not the tip cells, is thrown away.*

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DESCRIPTION OF PLATES.

All the figures were drawn with the camera lucida under Zeiss apochromatic lenses, Obj. 16mm., Occ. 18. The positions of all the cell walls and nuclei were represented with as great faithfulness as possible. With the exception of Figs. 69–73, Pl. VI, which represent ova of Crepidula plana, all the preparations figured are of C. fornicata. In Pls. II–VI the mesoblast is colored red, the enteroblasts (intestinal cells) blue, mesentoblast violet. In Pl. VII the violet indicates the nervous system. In Pls. VIII and IX yellow indicates the yolk cells, neutral tint ectoderm, and a darker shade of neutral tint mesoderm.

Reference Letters.

AB.	Anterior branch of velum.
AC.	Apical cell plate.
An. C.	Anal cells.
Ant.	Anterior.
Ap.	Apex.
Ap. O.	Apical organ.
as.	Aster.
Bl.	Blastopore.
CC.	Cerebral commissure.
CG.	Cerebral ganglion.
C. P.	Cerebro-pedal connective.
Dor.	Dorsal.
Ect.	Ectoderm.
Ent.	Entoderm.
EN.	Egg nucleus.
Ex. K.	External kidney (Urniere).
Int.	Intestine.
L.	Left.
Men.	Mesenteron.
Mes.	Mesoderm.
O.	Mouth.
Oc.	Ocellus.
O.M.	Opening between yoke cells into mesenteron.
Op.	Operculum.
Ot.	Otocyst.
P.	Foot.
PB.	Posterior branch of velum.
PC.	Posterior cell plate.
P. Cav.	Cavity of foot.
P. Com.	Pedal commissure.
PG.	Pedal ganglion.
pf.	Polar furrow.
Post.	Posterior.
Post. O. V.	Post-oral velum.
Pre. O. V.	Pre-oral velum.
PP.	Pedal cell plate.
R.	Right.
Sh.	Shell.
Sh. G.	Shell gland.
Sh. G. E.	Margin of shell gland.
SN.	Sperm nucleus.
St.	Stomodaeum.
T.	Tentacle.
UC.	Umbrella cavity (head vesicle).
V.	Velar ridge.
V.^{1}	First cell row of velum.
V.^{2}	Second cell row of velum.
I.	First cleavage plane.
II.	Second cleavage plane.
1st. pb.	First polar body.
2d. pb.	Second polar body.

A. Left anterior macromere.
B. Right anterior macromere.
C. Right posterior macromere.
D. Left posterior macromere.
1*a*, 1*b*, 1*c*, 1*d*, 1*a*^{1}, *etc.* First quartette of ectomeres.
2*a*, 2*b*, 2*c*, 2*d*, 2*a*^{1}, *etc.* Second quartette of ectomeres.
3*a*, 3*b*, 3*c*, 3*d*, 3*a*^{1}, *etc.* Third quartette of ectomeres.
4*A*, 4*B*, 4*C*, 4*D*. Fourth quartette (Entomeres and mesomeres).
5*A*, 5*B*, 5*C*, 5*D*. Fifth quartette (Entomeres).
ME, *ME*, *Me*, *Me*. Mesentoblasts.
M, *M*. Mesoblastic teloblasts.
E, *E*, *e*, *e*. Enteroblasts (intestinal cells).

EXPLANATION OF PLATE I.

FIG. 1. Egg of Crepidula fornicata, from side, showing male and female pronuclei in contact.

FIG. 2. First cleavage spindle, seen from above.

FIG. 3. Appearance of first cleavage furrow, seen from side. Daughter nuclei not yet reconstituted.

FIG. 4. Same egg as Fig. 3, seen from above.

FIG. 5. Completion of first cleavage furrow. Nuclei and asters opposite each other in the two blastomeres. Polar bodies between the two nuclei.

FIG. 6. "Resting stage" after first cleavage, showing flattening of blastomeres against each other and dexiotropic turning of nuclei, asters, and protoplasmic areas.

FIG. 7. Beginning of second cleavage. Laeotropic turning of spindles and protoplasmic areas.

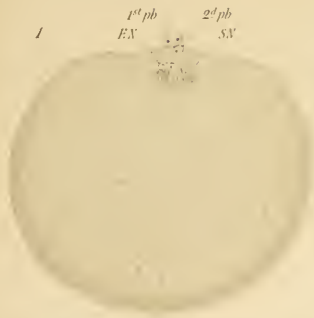
FIG. 8. Three-cell stage; an unusual condition, in which one of the first two blastomeres divides before the other.

FIG. 9. Second cleavage; usual condition, in which the two blastomeres divide simultaneously. Polar furrow appearing at *pf*.

FIG. 10. Completion of second cleavage. Asters nearly in position of the poles of preceding spindles. Polar furrow well formed.

FIG. 11. "Resting stage" after second cleavage. Four cells.

FIG. 12. Third cleavage. Spindles nearly radial.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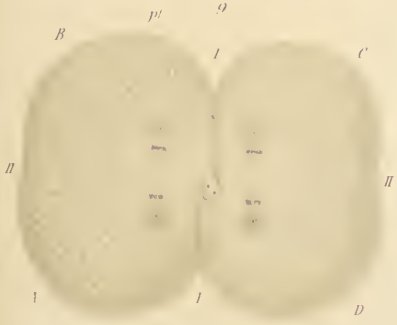
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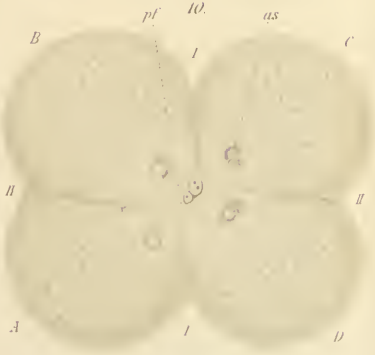
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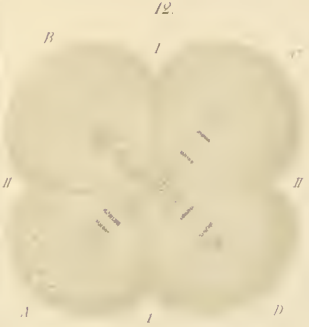
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EXPLANATION OF PLATE II.

FIG. 13. Completion of first quartette. Position of poles of preceding spindles as indicated by asters show that division was dexiotropic. Eight cells.

FIG. 14. Fourth cleavage, laeotropic. First quartette has rotated into furrows between macromeres.

FIG. 15. Completion of second quartette and laeotropic rotation of ectomeres. Twelve cells.

FIG. 16. Fifth cleavage. Laeotropic division of first quartette and formation of "turret cells" (trochoblasts), 1a, 1b, 1c, 1d.

FIG. 17. Sixth cleavage, dexiotropic. Formation of third and last quartette of ectomeres. Sixteen cells.

FIG. 18. First division of second quartette, dexiotropic. 3d has recently been separated from macromere D and still shows astral radiations. Twenty cells.

FIG. 19. "Resting stage," showing quadrangular plate of ectomeres with angles of plate in furrows between macromeres. Twenty-four cells, 4 apicals, 4 turrets, 12 belt cells, and 4 macromeres.

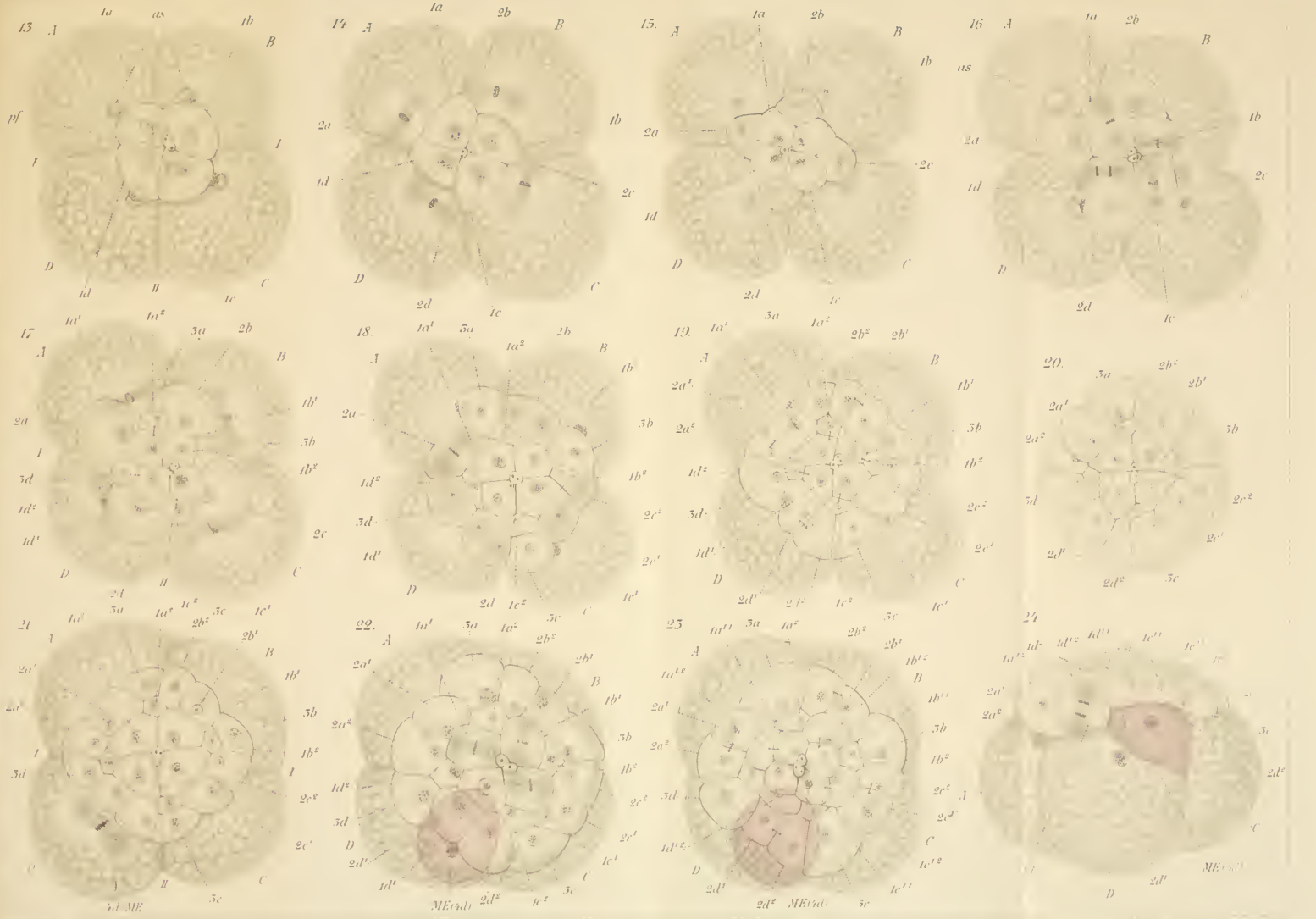
FIG. 20. Ectoblastic plate removed from macromeres, showing the overlapping of cells.

FIG. 21. Laeotropic division of D and formation of first member of fourth quartette, the mesentoblast 4d (ME). Twenty-four cells.

FIG. 22. Second division of first quartette (dexiotropic) and formation of basal cells of cross. The spindle has not yet appeared in 1d. Twenty-five cells.

FIG. 23. Complete separation of basal cells of cross ($1a^{1.2}$, $1b^{1.2}$, $1c^{1.2}$, $1d^{1.2}$). Twenty-nine cells.

FIG. 24. Side view of egg of about the same stage as Fig. 23, showing the relation of the mesentoblast to macromere D. 3d dividing.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EXPLANATION OF PLATE III.

FIG. 25. Dexiotropic division of the mesentoblast, 4D. The cell 3d is dividing almost radially. Twenty-nine cells.

FIG. 26. Separation of 4D into right and left halves. Radial division of 3c and 3b (3a divides a little later). Dexiotropic division of $2a^1$, $2b^1$, $2c^1$, $2d^1$ and formation of tip cells of cross ($2d^{1.1}$, $2c^{1.1}$, etc.).

FIG. 27. Same egg seen from the posterior side, showing position of right and left mesentoblasts and direction of spindles in some of the outer belt cells.

FIG. 28. Same egg seen from right side, showing direction of spindles in outer belt cells.

FIG. 29. "Resting stage" after the divisions shown in preceding figures. The cross is shown here and elsewhere in heavier outline. Forty-two cells, 4 apicals, 8 cross, 4 turrrets, 20 belt cells, 2 mesentoblasts, 4 macromeres.

FIG. 30. Bilateral division of mesentoblasts.

FIG. 31. Completion of cleavage shown in preceding figure and formation of enteroblasts, E^1 and E^2. *Dexiotropic* division of basal cells of cross, $1a^{1.2}$, $1b^{1.2}$ ($1c^{1.2}$ divides a little later). Forty-four cells.

FIG. 32. Completion of division of basal cells in arms a, b, and c, and formation of middle cells, $1a^{1.2.2}$, $1b^{1.2.2}$, $1c^{1.2.2}$. Second bilateral division of mesentoblasts. Forty-seven cells.

FIG. 33. Completion of division of mesentoblasts shown in preceding figure and formation of the primary mesoblast cells m^1 and m^2. Laeotropic division of the macromeres A, B, and C and formation of the remaining members of the fourth quartette, 4A, 4B, 4C. Laeotropic rotation of whole ectoblastic plate. Fifty-two cells.

FIG. 34. Side view of same egg, showing laeotropic formation of fourth quartette. Cf. Fig. 24.

FIG. 35. Division of $2c^{1.2}$, $2c^{2.1}$, $2b^{1.2}$, $2b^{2.1}$ ($2a^{1.2}$ and $2a^{2.1}$ divide immediately after). Flattening of cells 4A, 4B, 4C into furrows between macromeres.

FIG. 36. Completion of divisions shown in preceding figure. Bilateral cleavage of $3c^1$ and $3d^1$ ($3a^1$ and $3b^1$ divide later, see Fig. 38). Sixty cells.

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EXPLANATION OF PLATE IV.

FIG. 37. Anterior view of egg shown in Fig. 36.

FIG. 38. Dexiotropic division of $3a^1$ and $3b^1$. Division of $2d^{1.2}$ and $2d^{2.1}$ (the corresponding divisions in the other quadrants are shown in Fig. 36). Sixty-four cells.

FIG. 39. Posterior view of same egg.

FIG. 40. Anterior view of another egg of nearly the same stage, showing division of $3b^1$.

FIG. 41. Third bilateral division of mesentoblasts. Belated division of $2a^{2.1}$.

FIG. 42. Complete separation of mesoblast and entoblast. Formation of enteroblasts, e^1 and e^2, and of mesoblastic teloblasts, M^1 and M^2. Division of primary mesoblast cells, m^1 and m^2. Transverse division of the middle cells in the right, left, and anterior arms of the cross ; teloblastic division of the basal and terminal cells of the posterior arm. Division of $3b^2$ a little before the corresponding cells in the other quadrants. Sixty-eight cells.

FIG. 43. Anterior view of another egg of similar stage, showing the division of $3b^2$ and $3c^2$.

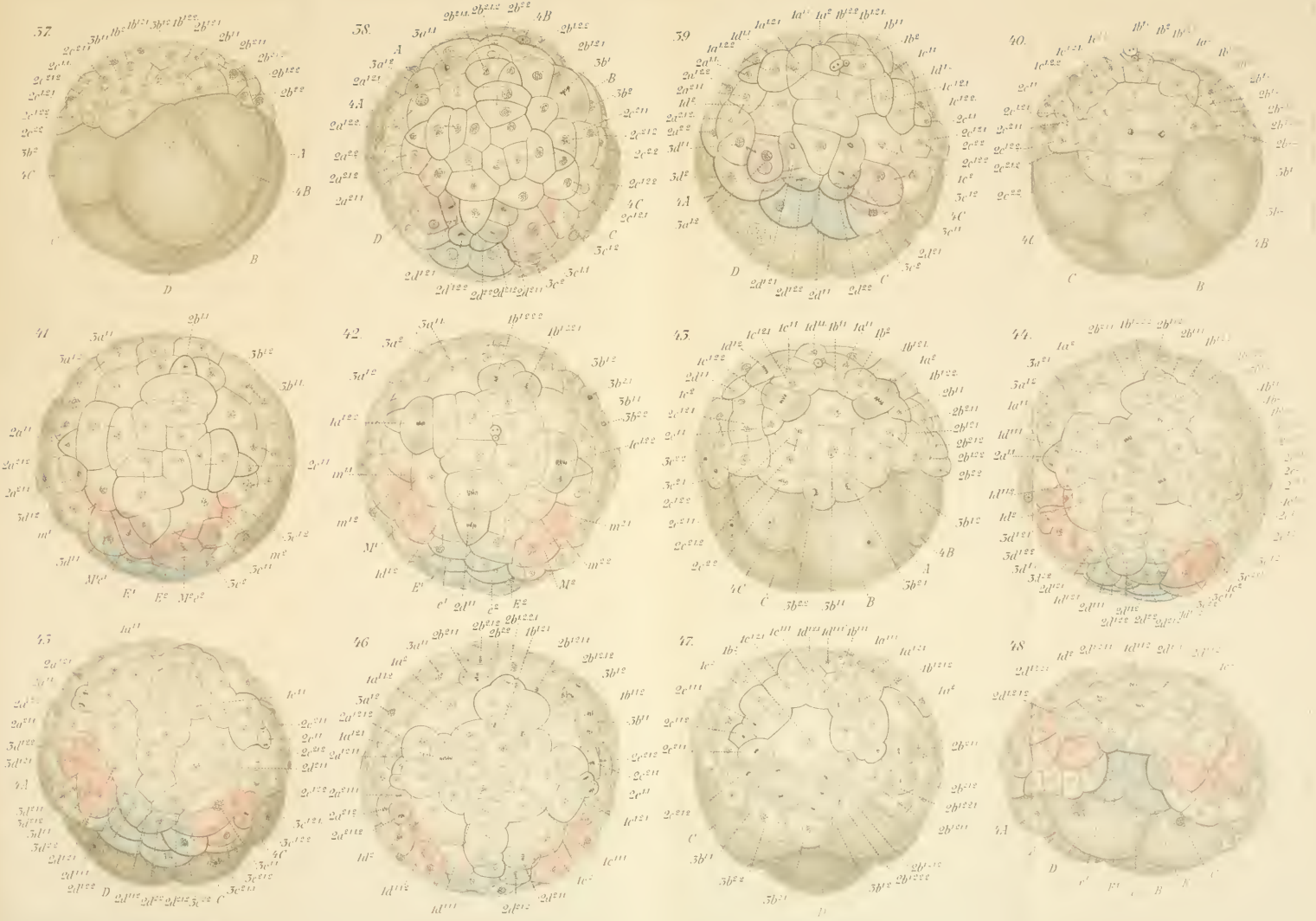
FIG. 44. "Rosette division" of the apical cells. Transverse division of the tip cells in arms a, b, and c. The cell $3c^{1.2}$ is dividing, $3d^{1.2}$ has divided. Eighty to eighty-six cells.

FIG. 45. Same egg seen from the posterior side.

FIG. 46. Transverse division of the basal cells in the arms a, b, and c. Nearly horizontal division of $2a^{2.1.1}$, $2a^{2.1.2}$, $2b^{2.1.1}$, $2b^{2.1.2}$, etc. Laeotropic division of $2a^{1.2.1}$, $2b^{1.2.1}$, etc. Dexiotropic division of $3b^{1.1}$, laeotropic division of $3b^{1.2}$ (the corresponding cells in the quadrant A divide soon after, $3c^{1.2}$ and $3d^{1.2}$ have already divided, $3c^{1.1}$ and $3d^{1.1}$ do not divide). One hundred and nine cells, — 4 apicals, 4 rosette, 4 turrets, 22 cross cells, 58 outer belt cells, 6 mesoblasts, 4 enteroblasts, 7 entoblasts.

FIG. 47. Same egg seen from anterior side, showing direction of spindles in outer belt cells.

FIG. 48. Posterior view of slightly older stage, showing teloblastic divisions of posterior tip cells ($2d^{1.1.1}$ and $2d^{1.1.2}$) and other cells of the same group (2d).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EXPLANATION OF PLATE V.

FIG. 49. Division of the right and left middle cells in the arms a, b, and c, and the expansion of these arms into a cell plate. The posterior arm is much elongated, and the posterior turrets and adjoining cells are much enlarged, forming the posterior cell plate. The apical cells lie anterior to the polar furrow.

FIG. 50. Apical view, showing the cross in heavy outline. The anterior turret cells ($1a^2$ and $1b^2$) have divided, and their products, together with those of $2b^{1.2.2.1}$, form a belt of six cells around the anterior side of the cross; this belt, together with the tip cells of the right and left arms ($2a^{1.1.1}$, $2a^{1.1.2}$, and $2b^{1.1.1}$, $2b^{1.1.2}$) form the first velar cell row, or prototroch. The posterior turrets ($1c^2$ and $1d^2$) are undivided. (See Note, p. 204.)

FIG. 51. Bilateral division of the outer rosette cells ($1a^{1.1.2}$, etc.), forming four "rosette series." The last stage in which the polar bodies remain attached. Continued anterior shifting of the apical pole.

FIG. 52. Ventral view of nearly the same stage as preceding, showing quadrangular blastopore, the enteroblasts in its posterior angle.

FIG. 53. Division of the right, left, and posterior apical cells and of the right and left basal cells in the transverse arms; the basal cells of the anterior arm have already divided.

FIG. 54. Ventral view of a slightly older stage, showing the narrowing of the blastopore and the division of the macromeres A and C.

FIG. 55. Dorsal view, showing great enlargement of the proximal cells of the posterior arm, and the continued forward shift of the apex.

FIG. 56. Apical view of about same stage as preceding figure. Cross shown in heavy outline. First velar row (prototroch) surrounds cross on anterior side.

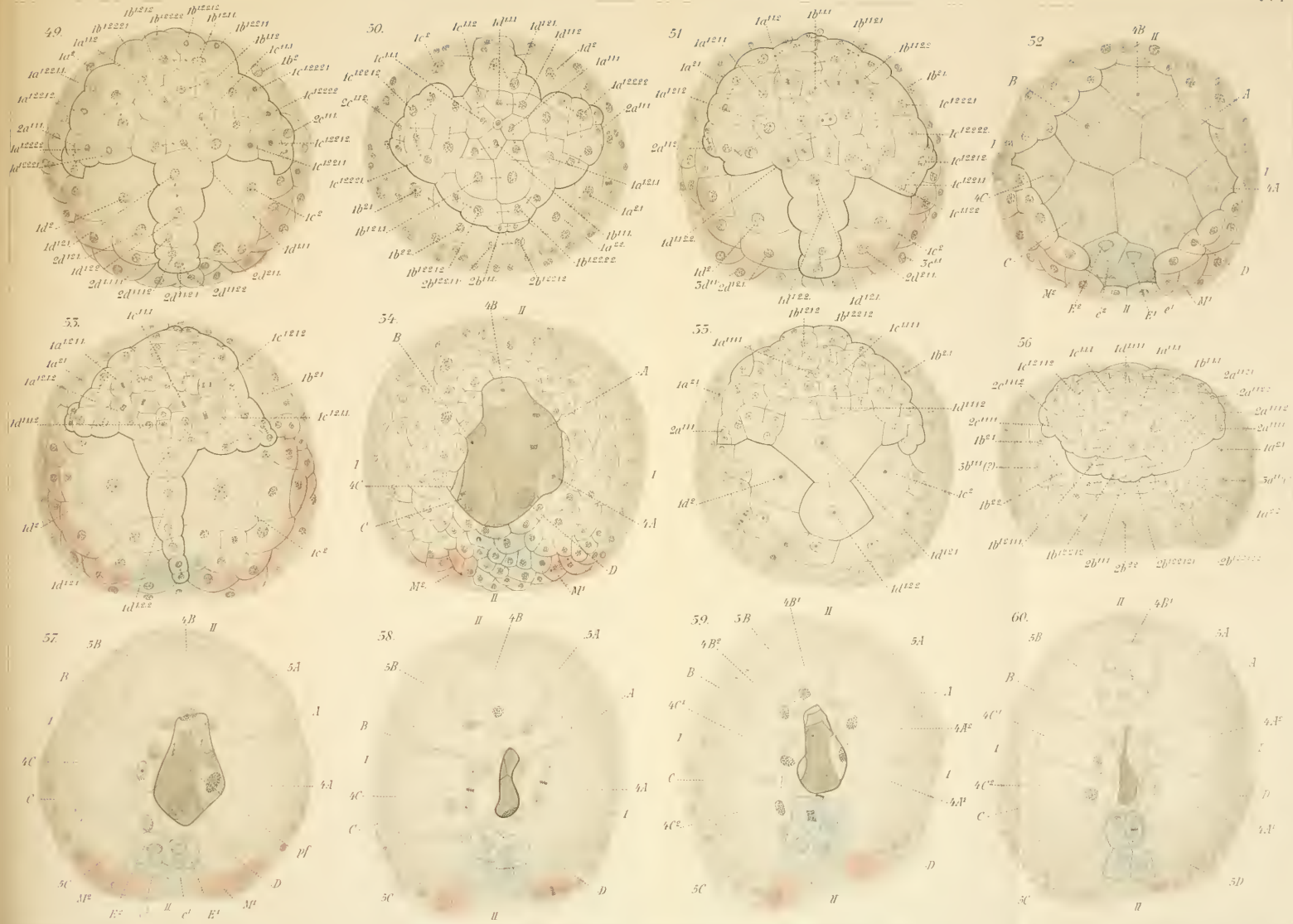
FIGS. 57–60. The ectoblast has been omitted from these figures in order to show more clearly the entoblast cells. The extent of overgrowth of the ectoblast is indicated by the margin of the blastopore.

FIG. 57. Formation of the fifth quartette; 5A and 5B on the ventral side of A and B, 5C posterior to C. Antero-posterior elongation especially on right side (left in figures).

FIG. 58. Fourth-quartette cells (4A and 4C) dividing. 4B divides immediately after.

FIG. 59. Fourth quartette (4A, 4B, 4C) divided; macromere D dividing. Turning of the posterior end to the left and beginning of final asymmetry.

FIG. 60. Completion of the 5th quartette by the formation of 5D, which lies opposite 5C. Division of the enteroblast E^1.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EXPLANATION OF PLATE VI.

FIG. 61. A stage after the formation of 5A, 5B, and 5C, but before the formation of 5D, showing the ectoblast in position. Mesoblastic bands hard to distinguish and not shown.

FIG. 62. Anterior view of egg of about same stage as Fig. 61. The apical cells, rosette series, and turret cells are shown. In this and the following figure the cells $2b^{1.2.2.1.1}$ (V′) and $2b^{1.2.2.1.2}$ (V′) should probably be designated $1b^{1.2.2.1.2}$ and $1b^{1.2.2.2.2}$, while the first velar row is probably formed, just ventral to these cells. (See Note, p. 204.)

FIG. 63. Narrowing of the blastopore.

FIG. 64. Dorsal view, showing apical cells, rosette series, posterior turrets, and proximal cells of posterior arm. The apex is far in front of the polar furrow.

FIG. 65. Closing of the blastopore and beginning of the stomodaeum. The apex has moved forward until it can be seen from the ventral side; it lies to the right (left in figure) of the mid line. Between the apex and the first velar row are the seven large cells of the apical cell plate. Both first and second velar cell rows are indicated. Many rows of cells radiate from the anal cells, running forward over the ventral surface posterior to the blastopore. The enteroblasts are turned to the right (left in figure) by the laeotropic torsion of the posterior region. In Figs. 65–73 the cells labelled $1b^{1.2.2.2.2.1}$ and $1b^{1.2.2.2.2.2}$ should probably be $1b^{1.2.1.2.2.1}$ and $1b^{1.2.2.2.2.1}$ respectively, and the arrows showing the derivation of these cells should be moved one cell nearer the apex so as to connect $1b^{1.2.1.2.1}$ and $1b^{1.2.2.2.2.1}$.

FIG. 66. Stage similar to the preceding, showing still more clearly the apical cell plate and the first and second velar rows.

FIG. 67. Apical view of same, showing apical cells, rosette series, posterior cell plate, apical cell plate, and first and second velar rows.

FIG. 68. Stage similar to the preceding, but showing in addition the stomodaeum, the shape and extent of the mesenteron, the division of the cell 5D and its rotation over to the ventral side, and the consequent looping of the intestine to the right (left in figure).

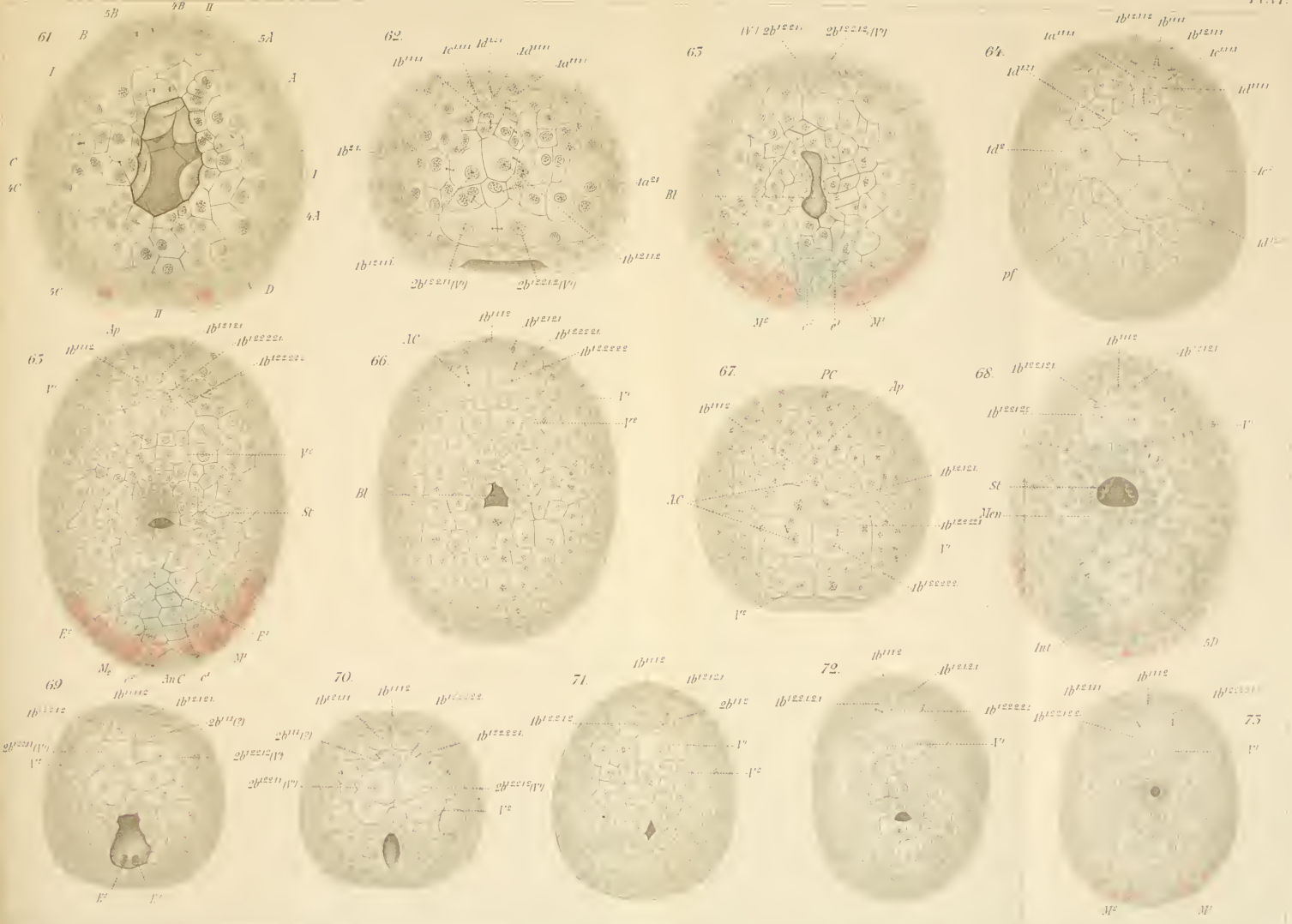
FIG. 69. Egg of C. plana, showing some of the cells of the apical cell plate and of the first and second velar rows. In Figs. 69–71 the cell marked $2b^{1.1.1}$ is probably $1b^{1.2.2.2.2.1.2}$ whereas the median cells of the first velar row (V′) probably represent the derivatives of the anterior tip cells ($2b^{1.1}$).

FIG. 70. Egg of C. plana, showing further stages in the degeneration of the cells marked $2b^{1.1.1}$ (?).

FIG. 71. Egg of C. plana, showing the earliest stage observed in the degeneration of the cells marked $2b^{1.1.1}$ and $2b^{1.1.2}$.

FIG. 72. Egg of C. plana, showing apical plate and velar cell rows.

FIG. 73. Egg of C. plana a little older than the preceding, showing apical plate, velar rows, mesoblast, and enteroblasts.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EXPLANATION OF PLATE VII.

FIG. 74. Dorsal view of an embryo of C. fornicata. The apical cells lie at the anterior margin of the figure, the dorsum is covered by the large cells of the posterior cell plate, the shell gland is forming on the postero-dorsal surface a little to the left of the mid line. The four macromeres and the polar furrow are still recognizable.

FIG. 75. Embryo similar to the preceding, but showing the invagination of the shell gland.

FIG. 76. Ventral view of an older embryo, showing the beginnings of the nervous system and the laeotropic torsion of the intestine. The foot is appearing as a prominent area posterior to the mouth.

FIG. 77. Side view of same embryo, showing the branching of the velum on each side of the posterior cell plate, and the relation of the intestine to the shell gland and foot.

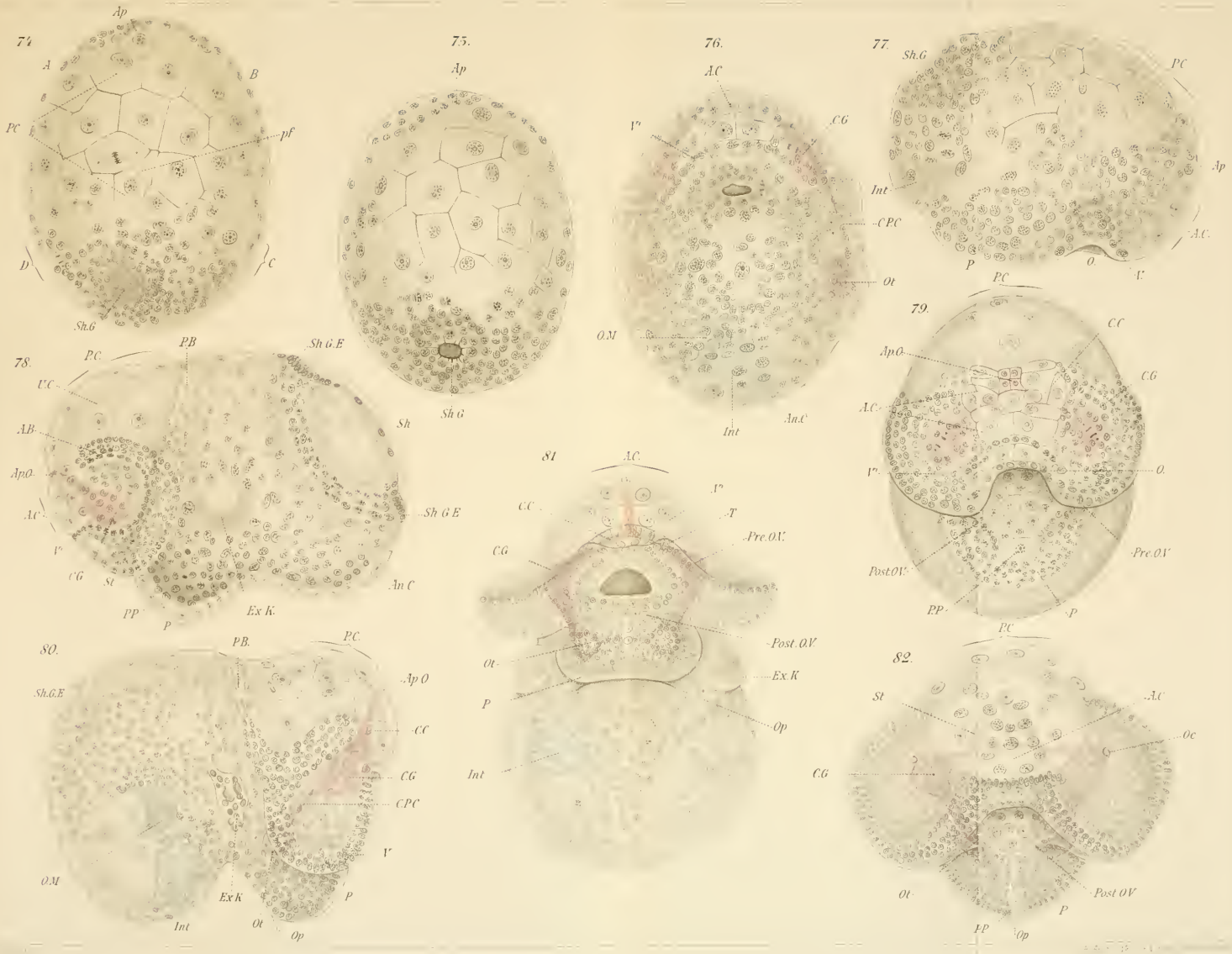
FIG. 78. Side view of older embryo, showing head and foot vesicles, apical plate and organ, cerebral ganglia and commissure, posterior and pedal cell plates, anterior and posterior branches of the velum, expanding shell gland, external kidney, stomodaeum and mesenteron.

FIG. 79. Apical view of same stage, showing apical, posterior and pedal cell plates, pre- and post-oral velum, apical organ and cerebral ganglia.

FIG. 80. Side view of older embryo. Intestine carried up on right side and opening at its proximal end into cavity between the yolk cells. Intestine surrounded by single layer of cells inclosing a clear cavity.

FIG. 81. Ventral view of similar embryo, showing circum oesophageal nerve ring, pre- and post-oral velum, etc.

FIG. 82. Apical view of still older embryo, showing pre- and post-oral velum, ocelli, otocysts, etc.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EXPLANATION OF PLATE VIII.

FIG. 83. Section of egg in four-cell stage, showing inclination of spindles in the formation of the first quartette.

FIG. 84. Section of egg similar to the one shown in Fig. 15. Formation of second quartette. Position of spindles not usually so nearly in the same plane.

FIG. 85. Section of egg similar to the one shown in Fig. 25. Extension of ectoblast over the yolk cells.

FIG. 86. Section of egg similar to Fig. 41 or 42. Further extension of ectoblast. Migration of nuclei and protoplasmic areas of the yolk cells in advance of ectoblast. Mesoblast cells seen in section.

FIG. 87. Transverse section of egg similar to Fig. 52.

FIG. 88. Vertical longitudinal section of embryo similar to Fig. 65 or 68. As the section is taken to one side of the mid line the opening from the stomodaeum into the mesenteron is not shown. Small yolk cells, derivatives of the fourth quartette, form the floor of the mesenteron.

FIG. 89. Median horizontal section through embryo of same stage as preceding, showing mesoblast and enteroblast cells at the posterior end.

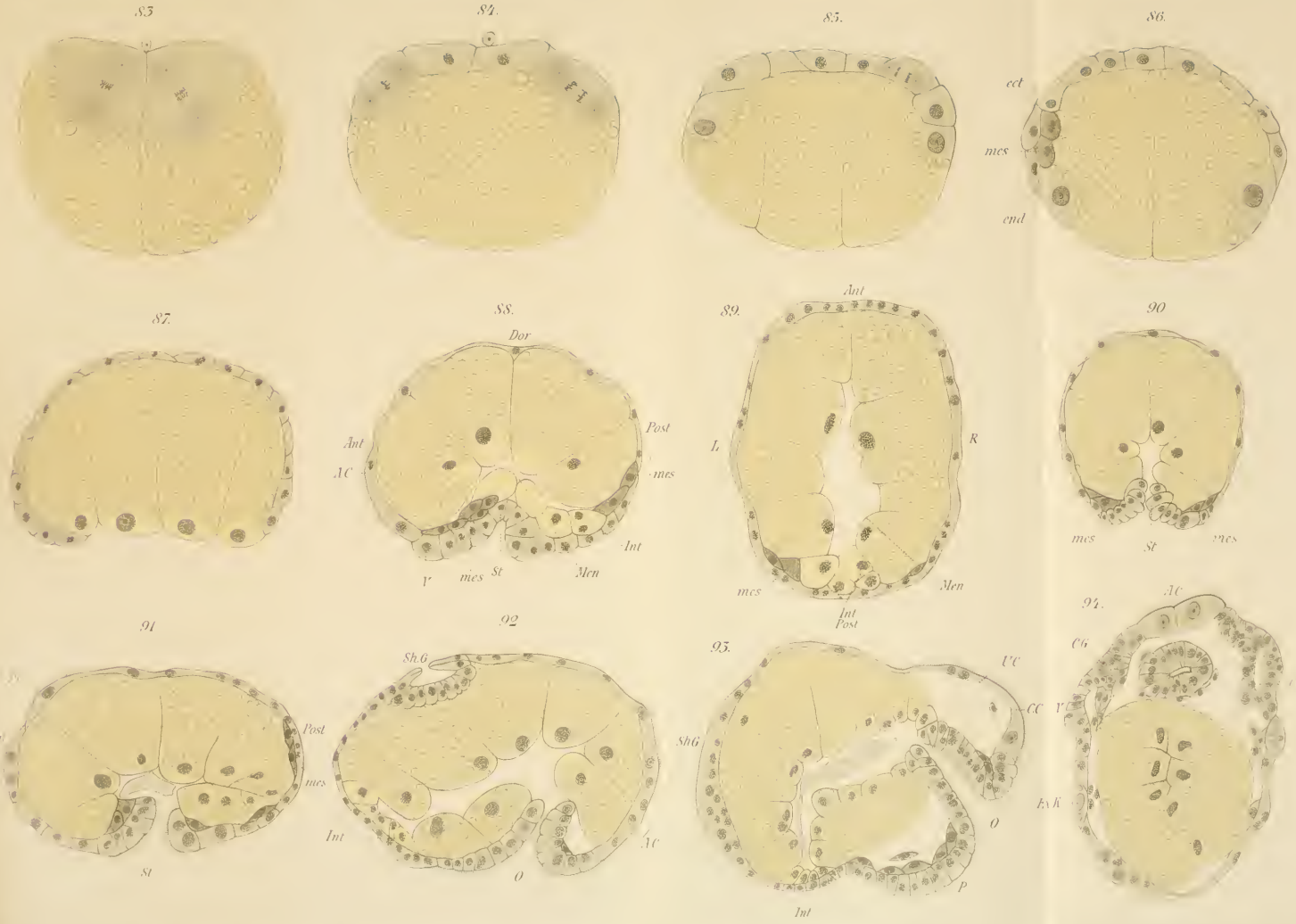
FIG. 90. Median transverse section of same stage as preceding, showing opening from stomodaeum into mesenteron. Here and elsewhere the smaller yolk cells form the floor and sides of the mesenteron.

FIG. 91. Vertical longitudinal section of stage shown in Fig. 74.

FIG. 92. Vertical longitudinal section of stage shown in Fig. 75, showing invagination of shell gland and formation of intestine.

FIG. 93. Oblique longitudinal section of stage shown in Fig. 78. At the posterior end the section lies to the right of the mid line, and hence passes through the opening from the intestine into the mesenteron. The shell gland has evaginated, and the intestine has been carried to the ventral side. By the growth of the foot the mouth has been carried far forward. The mesenteron contains a coagulum derived from the albuminous fluid in which the embryos are immersed in the capsules.

FIG. 94. Horizontal section of the same stage as the preceding taken in the direction of a line connecting the points CC. and Int. of Fig. 93. The formation of the cerebral ganglia and the cerebro-pedal connectives is shown.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EXPLANATION OF PLATE IX.

FIG. 95. Vertical longitudinal section, stage similar to Fig. 78. At the posterior end the section cuts the intestine as a tube (cf. Figs. 76 and 81).

FIG. 96. Coronal section of embryo of stage shown in Fig. 78, taken through apical organ, cerebral ganglia, and foot.

FIG. 97. Section from same series as preceding, two sections farther back, showing cerebro-pedal connectives and otocysts.

FIG. 98. Section similar to preceding, taken just posterior to the opening of the stomodaeum into the mesenteron.

FIG. 99. Horizontal section of embryo similar to Fig. 79, taken in the direction of a line connecting the points O and V^1 in that figure.

FIG. 100. Horizontal section of embryo similar to Fig. 76, showing formation of otocysts and intestine.

FIG. 101. Horizontal section of embryo similar to Fig. 80, taken a little above reference line V of that figure.

FIG. 102. Horizontal section of embryo similar to Fig. 80, taken near the dorsal side and through the intestinal cell plate which forms the dorsal boundary of the intestine at the point where it opens into the mesenteron (see Fig. 80).

FIG. 103. Section from same series as preceding, lying a little nearer the ventral side, and taken through the opening of the stomodaeum and of the intestine into the mesenteron.

FIG. 104. Horizontal section of an older embryo, taken nearer the ventral side than the preceding, about the level of the ocelli of Fig. 82.

FIG. 105. Section from same series as preceding, but nearer the ventral side, taken at the level of the otocysts in Fig. 82.

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THE EARLY DEVELOPMENT OF MARINE ANNELIDS.

A. D. MEAD.

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THE material for the preparation of this paper was procured at Woods Holl, Mass. The work has been carried on at the Marine Biological Laboratory, at Clark University, and at the University of Chicago under the direction of Dr. C. O. Whitman. I desire here to express my appreciation of the generous treatment which I have received at these institutions, and especially to thank Dr. Whitman for his interest and friendly counsel, which encouraged me to pursue these researches.

PART I. — DESCRIPTIVE.

A. AMPHITRITE ORNATA VERRILL.

Colonies of *Amphitrite* are found in several places in the vicinity of Woods Holl, Mass., being more abundant at Lackey's Bay, Hadley Harbor, and Ram Island. The animals live in tough mud-tubes, from just below low-water mark to a considerable depth. A little experience enables one to locate the tubes easily, for every one has two openings, each at the summit of a peculiar mound which resembles a small anthill. The capture of the worms themselves is attended, however, with a good deal of difficulty, since they retreat to a considerable depth beneath the surface of the mud. The limits of the breeding season are unknown. Although about eight hundred worms were collected, in lots of twenty or thirty, between the first of June and the last of August, only seldom were ripe eggs and ripe spermatozoa obtained. Naturally the eggs are not found in the sea, for they are just the color of the mud, and are doubtless discharged free into the water, and soon scattered by the currents.

The females are darker colored than the males, and the eggs can often be seen through the body wall. It is useless to cut the animals open, for, if the sexual products are mature, they will be discharged, usually at about 6 o'clock in the evening, more often on the day of capture, sometimes the next day. The rarity of ripe specimens is partly compensated for by the enormous number of eggs which may be obtained from one female. They may be kept in the sea-water for an hour or more without harm, and then successfully fertilized by simply adding the sperm, though it is necessary to rinse the eggs later to rid them of the superfluous spermatozoa. The late larvæ, five days old or more, are best kept in a dish with fresh ulva ; the trochophores soon lose their cilia and creep upon the sea-weed.

For killing, I have used Kleinenberg's picro-sulphuric (strong), chrom-formic, Perenyi's fluid (for surface views), and Flemming's fluid, weaker formula.

My first material was so damaged by the tannin which the alcohol extracted from the cork stoppers that I discarded the

latter and used plugs of absorbent cotton covered with thin linen paper to prevent losing the minute eggs in the cotton. The vials were then packed in glass jars with labels toward the outside, and the whole jar filled with strong alcohol.

In staining for surface views I have always obtained the best results with Delafield's hæmatoxylin, especially with Conklin's method of using the stain diluted and acidulated. The stained eggs are cleared in clove oil or cedar-wood oil in the ordinary way, and mounted in balsam, the cover glass being supported by four paper "feet." A little clove oil allowed to mix with the balsam keeps the latter soft, so that for weeks the egg can be rotated and drawn with the camera, in any position.

In drawing surface views with the camera lucida, when the cell outlines are faint and complicated, I have used thin black paper and a soft lead pencil, the point of which has been painted with *chinese white*. The advantages of this method are twofold; the image of the whitened pencil-point and that of the egg are both more distinct, and the pencil lines on the paper are never confused with the lines of the image. By simply smearing the back of the paper with soft lead and stub, the sketch may be easily transferred to drawing paper.

In the belief that it is unwise to depart from a nomenclature which involves nearly all the observations in this particular type of cleavage, unless a greatly superior one can be substituted, I have adopted that introduced by Wilson in his *Nereis* paper,[1] and employed with certain modifications by Heymons[2] and Lillie[3] (Kofoid's[4] ingenious system of naming the cells avoids possible objections to Wilson's system, but is fraught with certain practical disadvantages). I have, however, amended Wilson's scheme in the following points to adapt it to comparative description.*

[1] Wilson, E. B.: The Cell Lineage of Nereis. *Journ. of Morph.*, vol. VI, 1892.

[2] Heymons: Zur Entwicklungsgeschichte von Umbrella mediterranea. *Zeitschr. f. wiss. Zool.* Bd. 56, 1893.

[3] Lillie, F. R.: The Embryology of the Unionidae, A Study in Cell Lineage. *Journ. of Morph.*, vol. X, no. 1, 1895.

[4] Kofoid: On Some Laws of Cleavage in Limax. *Proc. Amer. Acad. Arts and Sci.*, January, 1894.

* Some of the points have been mentioned by Kofoid and Lillie.

(1) Instead of designating cells of *several successive generations* by the same letters, *e.g.*, the "macromeres" *A*, *B*, *C*, *D*, I have used subscripts, thus : A, A_1, A_2, etc. (2) Instead of naming the daughter cells sometimes from their relative position and sometimes from their relative size, I have named them uniformly with respect to their relative *position*, giving the cell

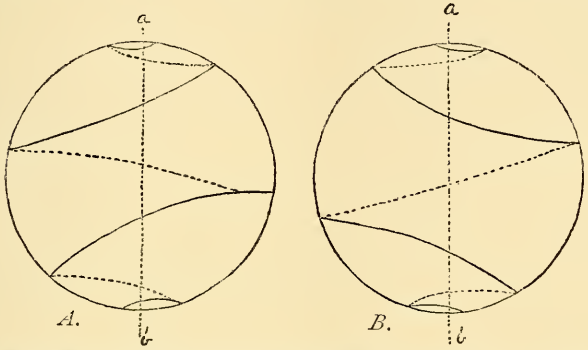

FIG. I. — Diagrams of egg with circumscribed loxodromic curves. *a-b*, axis. The curve in *A* represents the general direction of the cleavage furrows in *right oblique cleavage ;* in *B* that of the furrows in *left oblique cleavage*.

nearer the vegetative pole the larger exponent. For example, a^2 divides into $a^{2.1}$ and $a^{2.2}$, and the latter is nearer the vegetative pole.

This nomenclature is employed purely as an instrument of convenience, and I do not thereby commit myself to any implied theory of development.

When the cleavage plane of a dividing cell takes the general direction of a loxodromic curve described about the egg in the direction indicated in text Fig. I, *A*, the cleavage is designated as *right oblique ;* when in the direction of the curve in Fig. I, *B*, as *left oblique*. Similarly, we may speak of vertical and horizontal cleavages, when the furrow takes the direction of the meridian, or one at right angles to it.

I. CLEAVAGE TO 64 CELLS.

The normal fertilized egg of *Amphitrite* is nearly spherical and about 100μ in diameter, although there is considerable individual variation. It is enclosed in a much-wrinkled mem-

brane, and is perfectly opaque. The cleavage nucleus is slightly eccentric, lying nearer the polar globules, and is surrounded by a thick layer of protoplasm, while the yolk is mostly segregated at the vegetative pole. For descriptive purposes the position of the polar bodies indicates approximately the *animal pole* of the egg and the center of the *anterior hemisphere;* the point 180° from this is the *vegetative pole*, the center of the *posterior hemisphere;* the line joining the two points is the vertical axis of the egg.

When the first cleavage spindle is formed, the egg is slightly oval, and the spindle always parallel with the long axis, on the same level as the segmentation nucleus, and *nearer one end* of the egg (Fig. 1, Pl. X). At this time the egg is completely oriented with reference to the future cleavage furrows. The rate of cleavage is influenced directly by the temperature; in July the first polar globule appears in less than half an hour after fertilization, and the first cleavage furrow about thirty minutes later.

The first furrow is meridional (parallel with vertical axis), and appears all round the egg at about the same time, though it sinks in somewhat more rapidly on the anterior hemisphere. It divides the egg into two blastomeres of unequal size, *A–B* and *C–D* (Fig. 2), and in a few minutes these divide nearly simultaneously, though the larger (*C–D*) is sometimes a little in advance. The direction of the cleavage is always *left oblique;* the karyokinetic spindles, even when in the equatorial-plate stage, are inclined to the plane of the equator, and indicate the direction of the cleavage (Fig. 3). Consequently the cells *B* and *D* in the 4-cell stage lie somewhat lower (nearer the vegetative pole) than *A* and *C*, and meet in a "cross-furrow" (Figs. 4, 5); but the "precocious formation" of the cross-furrow found in some eggs, for example, in *Crepidula* and *Nereis*, is not apparent in *Amphitrite*. In the cleavage of the two blastomeres, as in the first cleavage, the furrows sink in more rapidly on the anterior hemisphere than on the yolk-laden posterior hemisphere (Fig. 4).

The cell *D* of the 4-cell stage (Figs. 4–6) is considerably larger than the other cells, and its descendants play a conspicu-

ous rôle in the development. Neither of the cleavage furrows coincides with the sagittal plane of the future embryo; this plane passes through the cells *B* and *D* (cf. Wilson,[1] p. 386).

All four cells undergo an almost synchronous right oblique cleavage, and an 8-cell stage results (Figs. 5, 6, 7). In consequence of the obliquity of the cleavage, the upper cells a_1, b_1, c_1, d_1 are not directly superimposed on the large ones A_1, B_1, C_1, D_1, but alternate with them. For convenience in description the cells a_1, b_1, c_1, d_1, or their descendants, will be regarded as constituting the *upper* or *anterior hemisphere*.

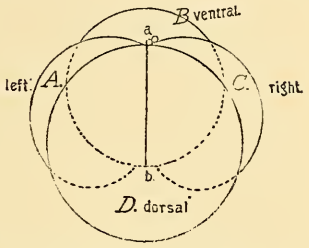

FIG. 11. — Diagram of 4-cell stage. *a*, animal pole; *b*, vegetative pole; *A*, *B*, *C*, and *D*, first four blastomeres, the anlagen respectively of the left, ventral, right, and dorsal quadrants of the trochophore.

The relative position of the upper and the lower quartette of cells is significant, for it alters the direction of the second cleavage furrow, twisting it so that its direction on the upper hemisphere is different from that on the lower, — a relation which is maintained throughout the subsequent cleavage. Each of the four cells has divided unequally, but with a remarkable result; on the posterior hemisphere in the 8-cell stage, D_1 is larger than A_1, B_1, C_1, which latter are about equal to one another in size, while on the anterior hemisphere, d_1 and c_1 are equal in size, but larger than a_1 and b_1. The four original cells have undergone a sort of *compensating division*, which establishes upon the *anterior hemisphere* a bilaterally symmetrical group of cells, whose plane of symmetry nearly coincides with the second cleavage furrow (Fig. 7).

The polar globules remain where they were formed, and lie near the meeting-point of the four upper blastomeres. There is already a small segmentation cavity (Fig. 7). The yolk is more abundant in the four lower cells, but the egg follows the same course of development whether in one position or another, and often has been observed to develop with the vegetative pole uppermost.

8–16 cells. — Except for slight variation, the eight cells cleave synchronously, and always obliquely to the left (Figs.

8–10). The sixteen cells (nearly ready to divide again) are seen in Figs. 11–14. In consequence of the regularity of the last cleavage, they are arranged in four alternating zones of four cells each. The cells of the second zone on the upper hemisphere, $a^{1.1}$, $b^{1.1}$, $c^{1.1}$, $d^{1.1}$, are the parent cells of the *primary prototroch* — "trochoblasts" (Wilson), so I shall call them *primary trochoblasts*. In *Amphitrite* these cells are more strictly "trochoblasts" than in *Nereis*, for *all their descendants* become prototrochal cells, while in *Nereis*, according to Wilson, some do not. The egg at this stage has a segmentation cavity of considerable size.

As we shall see below, certain cells of the lower hemisphere, a^2, b^2, c^2, also contribute to the prototroch in *Amphitrite*. d^2 (X), however, does not do so, but has a special destiny: it is to form the whole ectoderm of the trunk, including proctodœal cells and paratroch. Therefore I shall call it, after Wilson, the *somatoblast*.

16–32 cells, Figs. 11–16. — All sixteen cells soon cleave *obliquely to the right*, though not quite synchronously. The two largest cells, d^2 and D_2, divide first (Fig. 14); the cells of the anterior hemisphere next (or frequently at the same time); the remaining cells of the lower hemisphere last. Notwithstanding these slight time variations, a 32-cell stage is attained, which may be described as consisting of eight quartettes of cells alternating with one another from pole to pole (Fig. 16). The thirty-two cells are arranged as follows: $a_{1.2}$, $b_{1.2}$, $c_{1.2}$, and $d_{1.2}$ meet at the animal pole; in the outer angles between them lie $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, and $d^{1.2}$; alternating with these are four of the daughter cells of the trochoblasts, $a^{1.1.1}$–$d^{1.1.1}$; and next the remaining four, $a^{1.1.2}$–$d^{1.1.2}$. These are all the cells of the *anterior hemisphere*. Alternating with the last four cells, come $a^{2.1}$, $b^{2.1}$, $c^{2.1}$, and $d^{2.1}$ (= X), which, with the exception of $d^{2.1}$, contribute nearly all their substance to the completion of the prototroch, and may be called *secondary trochoblasts*. Next in order are the quartettes, $a^{2.2}$–$d^{2.2}$, a^3–d^3, and at the lower pole, A_3–D_3.

Up to this point, in the transition from one stage to another, all the cells divide almost simultaneously and in the same direction ; all the divisions are oblique, and the direction — to the right or left — alternates with each succeeding stage

(cf. "Law of Alternating Spiral Cleavage," Wilson,[1] Kofoid,[4] etc.). If the cleavage were to continue in this regular fashion, we should find each of the thirty-two cells dividing at about the same time, and the cleavage furrow cutting each cell obliquely to the left. The result would be sixty-four cells having the regular arrangement characteristic of the earlier stages. *Amphitrite* falls slightly short of the ideal, for some cells divide sooner than others, and the most precocious are ready to divide again, when the most tardy ones have divided once. This tendency was recognized in the earlier cleavage, 16–32 cells, and has now become accentuated (Pl. XI, Figs. 17–28). Except for this comparatively slight irregularity in time of division, the cleavage of all thirty-two cells takes place with the rhythmical regularity characteristic of the typical alternating oblique cleavage: the cleavage of every cell is oblique and to the left.

The transition from the 32 to the 64-cell stage is especially interesting, because it is the last instance of the rhythmical division of all the cells, and because by these divisions the prospective germ layers become completely segregated into special cells, and the primary prototroch differentiated. The order of division can be seen by consulting Figs. 17–28. As a rule, almost immediately after the completion of the 32-cell stage, D_3 divides into D_4 and d^4 (= M). The former is the smaller and is pushed outward in a peculiar manner so that it lies at first above the level of the other cells (Fig. 20), but later crowds in among them, and forms part of the entoderm plate. d^4 (= M) is the original *mesoderm cell*, and apparently gives rise to all the mesoderm of the body. Before this division is completed, the eight daughter cells of the *primary trochoblasts* prepare to divide (Figs. 17, 21, 22, colored brown). All the sixteen cells resulting from this cleavage acquire cilia, and constitute the *primary prototroch* (Figs. 18, 19). Meanwhile the four animal-pole cells divide almost simultaneously, but with many slight variations in different eggs. The innermost products, $a_{1.3}$, $b_{1.3}$, $c_{1.3}$, and $d_{1.3}$, constitute the "*apical rosette*," while the outermost are parent cells of another characteristic pattern, — the "*cross*" $a^{1.3}$, $b^{1.3}$, $c^{1.3}$, and $d^{1.3}$ (colored blue, Figs. 18, 24).

The two daughter cells of the somatoblast, *i.e.*, $d^{2.1}$ and $d^{2.2}$

(X_2 and x^2), are among the first to divide (Fig. 20, also blue). Hereafter I shall speak of these cells or their descendants as the *somatic-plate*, for they give rise in *Amphitrite* not only to the *ventral plate* of the trunk, but to the *lateral* and *dorsal* areas as well. The secondary trochoblasts also divide early: $a^{2.1}$, $b^{2.1}$, and $c^{2.1}$ (Figs. 21, 22).

Soon after the cleavage of the rest of the cells takes place. A_3, B_3, and C_3, whose products, A_4, B_4, C_4 and a^4, b^4, c^4, with those of D_4, give rise to the entoderm (stippled in figures), are among the last to divide.

It is essential to obtain a clear idea of the orientation at the ideal 64-cell stage, for with reference to it the future cleavage will be described, one group of cells at a time. In the following table the cells are arranged in groups from the animal to the vegetative pole:

Anterior Hemisphere	4	$a_{1\cdot3}$ $b_{1\cdot3}$ $c_{1\cdot3}$ $d_{1\cdot3}$	rosette	Ectoderm
	4	$a^{1.3}$ $b^{1.3}$ $c^{1.3}$ $d^{1.3}$	cross	
	8	$a^{1.2.1}$ $b^{1.2.1}$ $c^{1.2.1}$ $d^{1.2.1}$ $a^{1.2.2}$ $b^{1.2.2}$ $c^{1.2.2}$ $d^{1.2.2}$	intermediate cells	
	16	$a_1{}^{1.1.1}$ $b_1{}^{1.1.1}$ $c_1{}^{1.1.1}$ $d_1{}^{1.1.1}$ or ap^1–dp^1 $a_2{}^{1.1.1}$ $b_2{}^{1.1.1}$ $c_2{}^{1.1.1}$ $d_2{}^{1.1.1}$ ap^2–dp^2 $a_1{}^{1.1.2}$ $b_1{}^{1.1.2}$ $c_1{}^{1.1.2}$ $d_1{}^{1.1.2}$ ap^3–dp^3 $a_2{}^{1.1.2}$ $b_2{}^{1.1.2}$ $c_2{}^{1.1.2}$ $d_2{}^{1.1.2}$ ap^4–dp^4	primary prototroch	
Equator				
Posterior Hemisphere	8	$a^{2.1.1}$ $b^{2.1.1}$ $c^{2.1.1}$ $a^{2.1.2}$ $b^{2.1.2}$ $c^{2.1.2}$	secondary prot. cells	
		$d^{2.1.1}$ $d^{2.1.2}$	somatic-plate cells	
	8	$d^{2.2.1}$ $d^{2.2.2}$		
		$a^{2.2.1}$ $b^{2.2.1}$ $c^{2.2.1}$ $a^{2.2.2}$ $b^{2.2.2}$ $c^{2.2.2}$	ectoderm of lower hemisphere	
	8	$a^{3.1}$ $b^{3.1}$ $c^{3.1}$ $d^{3.1}$ $a^{3.1}$ $b^{3.1}$ $c^{3.1}$ $d^{3.1}$		
		d^4	mesoderm	Mesoderm
	8	a^4 b^4 c^4 A_4 B_4 C_4 D_4	entoderm cells	Entoderm
	64			

Summary.— The fertilized egg of *Amphitrite* is spherical, about 100μ in diameter, and covered with a wrinkled membrane. The first cleavage is unequal, and in the 4-cell stage one blastomere is larger than any of the others. A vertical plane passed through the larger blastomere and the one diagonally opposite corresponds to the sagittal plane of the future embryo. The number of cells increases in geometrical progression from one to sixty-four. From the 2 to the 64-cell stage every cleavage furrow is oblique to the meridian of the egg, and the direction of the obliquity regularly alternates with each succeeding cleavage. From the 8-cell stage onward we distinguish an anterior or upper hemisphere — the four upper cells or their descendants, and a posterior or lower hemisphere — the four lower cells or their descendants. In consequence of a slight rotation of one hemisphere upon the other, the second cleavage furrow more nearly coincides with the future sagittal plane in the upper than in the lower hemisphere. In the 64-cell stage the material for the several germ layers is completely sorted out so that one cell represents the future mesoderm, seven cells the entoderm, and the remaining fifty-six cells, the ectoderm. The latter fall naturally into groups, which from the animal pole are: (1) rosette, (2) cross, (3) intermediate cells, (4) primary prototroch, (5) secondary prototroch, (6) somatic-plate, (7) the rest of the ectoderm of the lower hemisphere. Then come the mesoderm and entoderm. The regularity of the cleavage ceases abruptly at the 64-cell stage, so that from this time one can best follow the cells in groups.

II. Later Cleavage to Formation of Paratroch.

a. *Anterior hemisphere.*

By the time the 64-cell stage is actually attained, the parent cells of the cross, $a^{1.3}$, $b^{1.3}$, $c^{1.3}$, $d^{1.3}$, contain karyokinetic spindles and soon divide, sometimes simultaneously, but often with slight differences in time (Fig. 24). The furrows always cut the meridian of the egg at right angles. *There is never a trace of the oblique cleavage characteristic of all the previous divisions.* The resulting cells form the pattern of a cross, with arms in-

clined at an angle of 45° to the median plane, while the *rosette* lies in the middle of the cross (Figs. 18, 24, 29, cross, blue). The rosette cells later divide obliquely to the right, thus continuing in the alternating cleavage (Fig. 30). The intermediate cells, those in the angles between the arms of the cross, all divide in the same direction — right oblique — though not all at the same time. As a general rule, the larger ones divide first, the smaller last (Fig. 30).

The sixteen cells of the primary prototroch *never divide again*, but are flattened down so as to present a very even surface, and soon become covered with cilia.

The succeeding divisions of the cross cells are of great interest. They are exactly bilateral, so that the divisions on one side are the mirrored image of those on the other (Figs. 30–36). First, the distal cells in the dorsal arms of the cross divide, $c^{1.3.2}$, $d^{1.3.2}$; but, before these divisions are completed, spindles appear in the corresponding cells of the ventral arms, $a^{1.3.2}$, $b^{1.3.2}$, and in the *proximal* cells of the dorsal arms, and soon also in the proximal cells of the ventral arms, $a^{1.3.1}$ and $b^{1.3.1}$. When all these divisions are completed, each arm of the cross has three cells in a row and an extra one at the base. *The cross is not only symmetrical with respect to the median sagittal plane, but nearly so with respect to a plane at right angles to this.* So far, and even farther, the cross in *Amphitrite* is exactly comparable to that in *Nereis* (cf. Wilson,[1] Pl. XVI, Fig. 40). The middle cells in the dorsal arms, corresponding to the "nephroblasts" in *Nereis*, never divide again, and, except for a small area left at the surface, are covered over by the surrounding cells, and become the huge *dorsal umbrellar mucous glands* (Figs. 31–37, text Figs. XI–XVII, *gl.l.* and *gl.r.*, p. 257). The striking similarity between the dorsal and ventral arms of the cross at once suggests that the middle cells in the ventral arms have a destiny similar to that of *gl.r.* and *gl.l.* — Unlike the dorsal cells, they each divide once meridionally, but this I think is their last division (Fig. 33). They are soon partially covered over like the corresponding cells in the dorsal arms. Since two pairs of unicellular mucous glands occupy a position in the later larva exactly corresponding to these two pairs of

cells (*gl.*, text Figs. XI–XVII), it seems extremely probable that the middle cells in the ventral, as well as in the dorsal arms of the cross, give rise to unicellular mucous glands. The outlines of the *cross* are destroyed by the further cleavage of its component cells (Figs. 36, 37). These later divisions are interesting because they are manifestly bilateral, and because they bear a remarkable correspondence to the same divisions in *Nereis*, as far as the latter are figured (*Nereis*,[1] Figs. 41–44).

Of the *intermediate cells* only those between the dorsal arms of the cross have been followed through several generations. My object in following these was to ascertain what cells, if any, migrate from the anterior to the posterior hemisphere, through the mid-dorsal interruption of the prototroch, that is, through the gap between the prototrochal cells of the *d* and *c* quadrants. In the 64-cell stage these cells are two in number, $d^{1.2.1}$ and $d^{1.2.2}$ (Figs. 18, 24, 29). The latter is much larger and divides first, obliquely to the right (Fig. 30), and the former divides in the same direction (Fig. 45, $d^{1.2.1}$).* The posterior product of $d^{1.2.2}$ divides and for the three descendants of $d^{1.2.2}$ we will substitute the letters, $l.^1$, $l.^2$, and $l.^3$ (Figs. 32, 34–36, 45). Next $l.^1$ divides (Fig. 51) and then $l.^3$, making five cells in the group (Fig. 54). By this time the interruption in the prototroch has become much narrowed by the approach of the huge prototrochal cells from either side, and the *l.* group is seen to have taken a position *below the narrowest part* (Figs. 58, 60). Meanwhile the anterior product of $d^{1.2.2}$ divides. One of the daughter cells is extremely minute, has a deeply staining nucleus, and serves as an excellent landmark (asterisk, Figs. 35, 36, 51, 54, 58, 59, 60). When the prototrochal cells have met in the middle line, this minute cell with its twin, which also has a peculiar appearance, lies just above the suture (Fig. 60).

Therefore, returning to Fig. 24 or 29, I think we are warranted in saying that about half the cell $d^{1.2.2}$ and possibly part of $d^{1.2.1}$ contribute to the structures posterior to the pro-

* In the figures, the progeny of $d^{1.2.1}$ are indicated by the heavy outlines of their nuclei, while the posterior product of the division of $d^{1.2.2}$ (and its descendants) are distinguished by *shaded nuclei* (Figs. 34–36).

totroch. It is obvious that, when the prototroch has united dorsally, communication is shut off between the general ectoderm of the two hemispheres.

I have not observed any further divisions of the rosette cells, now eight in number, and believe that they bear the apical tuft of cilia, which appears long before the interruption of the prototroch is obliterated.

b. *Posterior hemisphere.*

Completion of prototroch. — Shortly after the 64-cell stage, the *secondary trochoblasts*, $a^{2.1.1}$, $a^{2.1.2}$, $b^{2.1.1}$, $b^{2.1.2}$, $c^{2.1.1}$, $c^{2.1.2}$, all divide obliquely to the right in accordance with the alternating rhythm (Figs. 26, 27, 28).

These divisions are precisely alike in each of these three quadrants, as shown in text Fig. III. $a^{2.1.1}$, $b^{2.1.1}$, and $c^{2.1.1}$ divide about equally, the others, $a^{2.1.2}$, $b^{2.1.2}$, $c^{2.1.2}$, very unequally. Of the resulting four cells in each group, the three larger ones are the *secondary prototrochal cells*, which never divide again, but soon become covered with cilia and form *part of the prototroch*. By virtue of their position they fill the gaps in the *primary prototroch*, excepting, of course, the mid-dorsal one, which, as we have seen, is filled by the concrescence of the primary prototrochal cells. By the addition of these nine cells, the prototroch is completed, and its twenty-five component cells may all be recognized even after the larva has begun to elongate (Figs. 57, 59, 61–63, and text Figs. V, VI). The outlines can always be seen, though difficult to follow in later stages.

Fig. III. — Diagram showing the relation of the cells in the region of the *secondary trochoblasts* just before the completion of the prototroch. It applies to the *A*, *B*, and *C* quadrants in both *Amphitrite* and *Clymenella*. The lightly stippled cells p^1 p^2, etc., belong to the primary prototroch; the heavily stippled cells (from the secondary trochoblasts) complete the prototroch.

Somatic-plate cells. — In the *posterior quadrant* $d^{2.1.1}$ and $d^{2.1.2}$ ($= X_2$ and x^2) behave in a manner very different from that of

the corresponding cells in the other quadrants. They divide somewhat in advance of the latter, and though $d^{2.1.2}$ ($= x^2$) divides in the same direction as the corresponding cells in the other quadrants, *i.e.*, continues in the alternating rhythm, $d^{2.1.1}$ ($= X_2$) does not, but divides somewhat obliquely in the *other direction* (Fig. 38).

The other somatic-plate cells, $x^{1.1}$ and $x^{1.2}$, divide again; $x^{1.1}$ synchronously with the division just mentioned (Fig. 38), but $x^{1.2}$ considerably later (Figs. 41–44).* Both these divisions are clearly contrary to the rule of alternating cleavage, for both are in the *same direction as that of their parent cell* x^1 (Fig. 20).

A glance at Figs. 38–40 is sufficient to show that the somatic-plate cells begin early to arrange themselves symmetrically with regard to the middle line of the embryo: X_3 is squarely in the middle line, x^3 nearly so (see future divisions); x^2 on the left side balances $x^{1.1}$ on the right and divides symmetrically with it. $x^{1.2}$, however, does not have a corresponding cell on the other side. It is worthy of note that in many animals this small cell arises in the same way, and has about the same proportional size (Unio,[3] Fig. 41, *Nereis*,[1] Fig. 55, *Clymenella*, Fig. 78, *Chætopterus*, Fig. 30).

I have watched the cleavage of the somatic plate from two principal points of view: that of developmental mechanics, and that of the axial relationships, the shifting of areas, etc.

The bilateral divisions of the somatic plate. — We have seen that up to the 64-cell stage all the divisions took place strictly in accordance with the rule of alternating oblique cleavage, with no regard to bilaterality. But after this stage the cells of the somatic plate divide *without regard to the rule of alternating cleavage, and with marked bilaterality*. Of the two divisions described last, that of x^2 might possibly be considered as a continuation of the alternating cleavage (Fig. 38). The division is oblique and to the right ; but since it has its mirrored image in the division of $x^{1.1}$, I believe, to state it paradoxically, that x^2 divides obliquely to the right for the same reason that $x^{1.1}$ *does not* — to conform to bilateral symmetry (Fig. 38, text Fig. IV).

* I have seen this cell in process of karyokinesis many times.

The cell X_3 divides meridionally and bilaterally, and the resulting cells, equal in size, lie one on either side of the

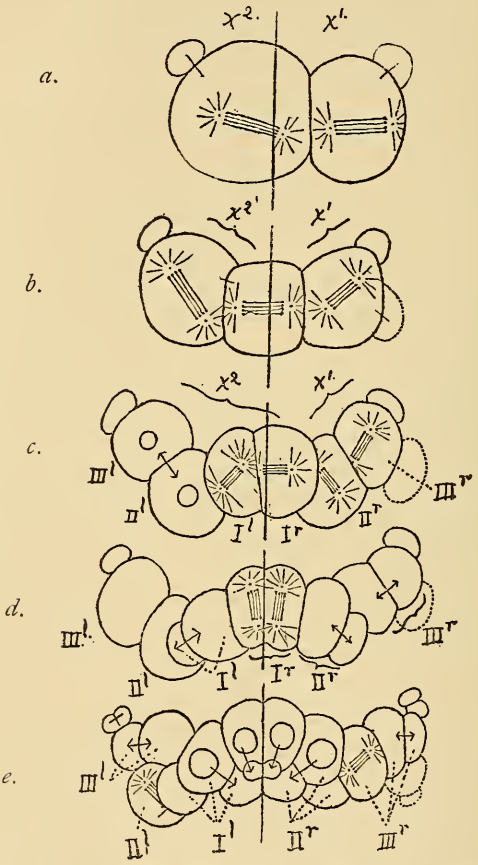

FIG. IV. — Diagram of a group of somatic-plate cells. The perpendicular lines indicate the sagittal plane of the trochophore. Figs. *a* aud *b* show the origin of the bilateral group of cells in *c*, where I^l, II^l, III^l on the left correspond in position to I^r, II^r, III^r on the right, although the sagittal plane passes through I^r. After the subsequent divisions the gronp is still bilateral, but the descendants of the cells I^l, II^l, and III^l no longer correspond in position to those of I^r, II^r, and III^r. The four cells in *e*, whose nuclei are indicated, constitnte the paratroch.

median line of the embryo. The later divisions of these cells are strictly bilateral as far as I have followed them (Figs. 39, 40–52, cf. p. 245).

The succeeding bilateral divisions of $x^{2.2}$ and $x^{1.1.2}$ are of special interest, for, although the cells, as a group, are not placed quite bilaterally with respect to the embryo, their next division corresponds in direction (Figs. 41, 42 and text Fig. IV, *a*, *b*). The products of $x^{2.2}$ are subequal, and of about the same size as the adjacent larger product of $x^{1.1.2}$, the latter having divided unequally. This division gives the impression of an effort towards bilateral cleavage in cells not perfectly balanced with respect to the middle line. As a result, the three subequal cells lie in a transverse row, one in the median plane. The small cell on the extreme right apparently has little to do with the balance of symmetry: it has no fellow on the left side (Figs. 41, 42 and text Fig. IV, *b*).

The middle cell of the group divides as an unpaired median cell usually does in bilateral cleavage, *i.e., meridionally and equally;* the cells on either side of it divide bilaterally — the division of either being the mirrored image of that of the other. As a result, we have six cells arranged in a bilaterally symmetrical group, *whose plane of symmetry falls slightly to one side of the middle line of the embryo* (Figs. 43–47, text Fig. IV). This being the case, it is obvious that, if the next division should take place *symmetrically with reference to the middle furrow of the group, the arrangement of the resulting cells would be asymmetrical with reference to the middle line of the embryo.* And conversely, if, after the next division, the cells should be arranged *symmetrically with regard to the middle line of the embryo, they must have divided with total disregard of the symmetry of the group.*

Which course will the cleavage pursue? As compared with the bilateral cleavage in eggs like the squid and ascidians, where the plane of symmetry of a group of cells coincides with that of the embryo, and where the origin of the cells on one side is the same as that of the corresponding cells on the other, the case we have in hand is very complex. — Here we have a bilaterally symmetrical group of cells somewhat asymmetrically placed, and the origin of the cells on one side is different from that of the corresponding cells on the other; they do not even belong to the same cell generation. To my mind this is a

pretty severe test of the influence of bilaterality of the whole organism upon the cleavage in certain parts. The two subsequent cleavages are therefore of special interest, for they give to the question an unequivocal answer. The next division takes place with *total disregard of the symmetry of the group*, and results in a new arrangement of the cells, which is *symmetrical with regard to the middle line of the embryo* (Figs. 46–52 and text Fig. IV, *c*, *d*).

The middle cells I^l and I^r are sister cells, equal in size and symmetrically placed in the group, but they divide very differently (text Fig. IV, *c*): I^r divides like an *unpaired median cell* and its equal products lie one on either side of the median line of the embryo; I^l divides symmetrically with II^r; III^r divides so that its products correspond to the (undivided) cells II^l and III^l. The grouping is thus rearranged, a new symmetry established, and the middle line of the new group coincides with that of the embryo. This is shown to be true, not only by the position of the cells, but by their subsequent divisions: corresponding cells *divide symmetrically* — bilaterally (Figs. 49–55 and Diagram III, *d*, *e*).

The four larger products of the four median cells are the *paratrochal cells*. They never divide again, and constitute the perianal paratroch of the trochophore, which persists as a ring of ciliated cells around the body just in front of the anus, until the larva has developed five or six metameres (Figs. 53–59, 61–64, light brown, text Fig. IV, *c*, with nuclei). The smaller products of these cells lie within the arc formed by the paratrochal cells and give rise to those structures only, which are posterior to the paratroch,—the proctodœum, etc. Since they mark the *posterior end* of the larva, I have called them *terminal cells*.

The following facts in regard to the origin of the paratrochal cells and the enclosed terminal cells, though obvious, are too important to be left unmentioned:

(1) *They all arise from the somatoblast X.* (2) *They lie at the posterior lip of the blastopore or entoderm plate.* (3) *At first the four lie nearly in a straight row, only the two dorsal ones meeting in the middle line.* (4) *Three of the four are*

descended from x^2, the remaining one (right ventral) from x^1: in other words, the material of three of the cells was separated from that of the fourth, at the first division of the somatoblast X (d^2, 16-cell stage), and, as we have seen, has had a different experience. (5) *Notwithstanding the differences in their past history, the four cells are symmetrically located and are of the same generation (11th generation remote from the ovum).*

To return to the somatic plate at the 64-cell stage—X_2 divides to form x^3 and X_3: X_3, the large cell, divides into two equal cells, lying one on either side the middle line; X_{3r} and X_{3l}, and their products divide bilaterally, and are symmetrically placed, Figs. 43–55; X_3 and X_3 divide into X_4 and X_4, and x^α and x^α; X_4 and X_4 into X_5 and X_5, and x^β and x^β; X_5 and X_5 into X_6 and X_6, and x^γ and x^γ. After this x^3 divides very unequally into $x^{3.1}$ and $x^{3.2}$; then X_6 and X_6 divide into X_7 and X_7, and x^δ and x^δ. At about this time x^β and x^β, x^γ and x^γ, and $x^{3.2}$ all divide bilaterally and equally.

The cleavage of the remaining ectoderm cells in the posterior hemisphere. — We have already described the fate of the secondary trochoblasts, page 240, and showed that, with the exception of three cells not much larger than polar globules, all their descendants enter into the prototroch (cf. the latero-dorsal trunk region in *Nereis*,[1] pp. 419, 427).

The remaining ectodermal cells fall naturally into groups; those descended from $a^{2.2}$, $b^{2.2}$, $c^{2.2}$, and from a^3, b^3, c^3, and d^3 of the 32-cell stage, — we have already described the division of each of these cells in the left oblique direction. There is little to be gained from a detailed description of the further cleavage of $a^{2.2}$, $b^{2.2}$, and $c^{2.2}$, though I followed it in the eggs in order (1) to be sure of the boundaries of the groups, (2) to become acquainted with any landmarks which might develop, (3) to see whether in these late stages there is the same constancy in the direction of division and in the size of the blastomeres in different individuals, that obtains in the earlier stages, and (4) to obtain data for close comparison with other species. (1) In Fig. 52 the boundaries of these groups are indicated. (2) The rows of small cells with deeply staining nuclei, not far from the edge of the somatic plate, are important landmarks

(Fig. 57). (3) In the late stages of cleavage, as in the early ones, all individuals of the species are alike. (4) The upper product of $a^{2.2}$, which forms the larval mesoblast in *Unio*, is in *Amphitrite* minute (Figs. 41–44), but its exact fate is unknown. The lower products of $a^{2.2}$, $b^{2.2}$, $c^{2.2}$ come to lie in about the same position as the "stomatoblasts" in *Nereis*, but I do not know their ultimate destiny. In each of the three groups, even at a late stage, one or two of the cells are of comparatively large size, and the rest very small; the former correspond in position to Wilson's "stomatoblasts" (Wilson,[1] p. 414).

In reference to the products of a^3, b^3, c^3, a^3, it need only be said that they behave in the same manner in all individuals, through several generations, and form important landmarks (cf. Figs. 41–62).

Entoderm. — At the 64-cell stage the entoderm consists of seven cells, which occupy a large part of the surface on the posterior hemisphere. They are A_4, B_4, C_4, D_4, and a^4, b, c^4, (d^4 being the mesoderm cell). The four cells first mentioned all divide again, and except for D_4, which divides obliquely to the right, and in advance of the others, they all cleave practically in the same direction as did the parent cells. The relative size and position of the resulting cells is remarkably constant in every egg. In consequence of these divisions the *entoderm plate* assumes the form of a cross, consisting of eleven cells arranged in the following manner:

$$d^4 = M - M = d^4$$

$$d^5$$

$$a^4 \quad a^5 \quad A \;\rangle\!\!\frac{D_5}{B_5}\!\!\langle\; C_5 \quad c^5 \quad c^4$$

$$b^5$$

$$b^4$$

The surface area of the cross gradually diminishes as the cells elongate at right angles to the surface.

None of these cells divide again until after they have migrated into the segmentation-cavity, where they give rise to the entoderm of the larva (Figs. 43–56, text Fig. VI).

Mesoderm. — The mesoderm cell $d^4 = M$, of the 64-cell stage, divides very early into two equal cells bilaterally placed upon

the surface (Fig. 38). These two cells, the anlagen of the paired mesoderm bands, immediately begin to elongate, and soon become cylindrical in shape. The nucleus of each migrates inward, and when it nears the middle of the cell, forms a karyokinetic spindle, and a very minute cell, *m*, is "budded

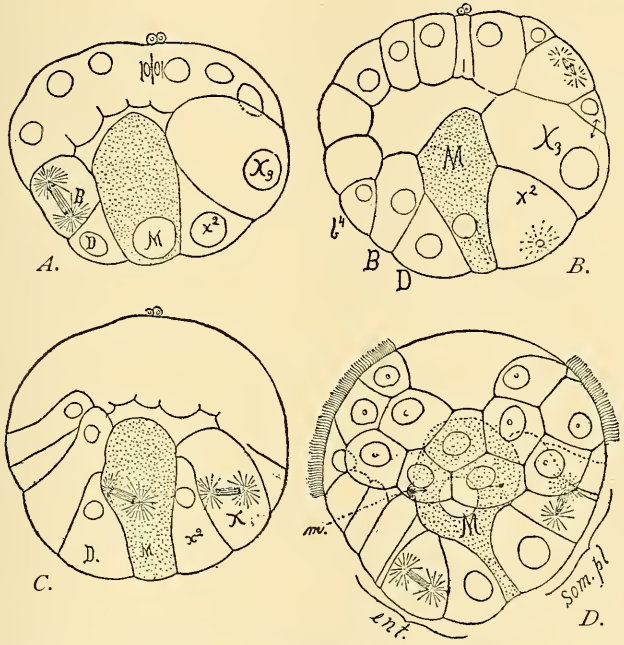

FIG. V. — Optical sagittal sections from camera sketches, showing invagination of the mesoderm. *M*, mesoderm; *m*, the minute cell, product of the division represented in *C*; *D*, *B*, *b*⁴, ent., entoderm; X_3 X^2, som. pl., somatic plate. In *D* the left half of prototroch is represented; those cells in which the nucleoli are drawn belong to the *primary* prototroch, the rest are the secondary prototrochal cells.

off" ventrally. These two small cells seem to correspond to those arising by the first division of the *M* and *M* in *Nereis*, *Unio*, and other forms. The peculiar thing about the division in *Amphitrite* is, that instead of taking place on the surface, it occurs after the mesoderm cells have begun their inward migration, but before they are wholly inside the segmentation cavity, and the spindles lie in the *short diameter of the cells*. Furthermore, the mesoderm cells, just at the time when the division

occurs, are squeezed in between the large somatic-plate cells on the dorsal side, and the large entoderm cells on the ventral side. In fact, no other cells seem to be under greater pressure at any period of development, and yet the axes of the spindles lie in the direction of greatest pressure (Figs. 41, 44, text Fig. V, *c*).

The two larger cells can be seen at the surface for a time after this division, but soon disappear within the segmentation cavity (Figs. 41–44, 46, 50). Once inside, they quickly assume a spherical form, having the minute cells *m* and *m* still attached to them (Fig. 50), and meet in the median plane. They behave like teloblasts, and give rise to the two diverging rows of mesoderm cells which, considerably later, break up into bands several cells wide. The cells *m* and *m* can be plainly distinguished until the mesoderm cell-rows contain five or six cells each (Fig. 52).

Summary. — With the attainment of the 64-cell stage the rhythmical, alternating, oblique cleavage suddenly stops. Some cells never divide again, some divide bilaterally, some continue to divide in the alternating oblique direction.

The formation of the apical rosette and cross is remarkably similar to that in *Nereis limbata*.[1] The middle cells of the dorsal arms of the cross, which become the "head kidneys" in *Nereis*, become large mucous glands in *Amphitrite*, while the corresponding cells of the ventral arms probably have a similar destiny. The sixteen cells which are the descendants of the *primary trochoblasts* $a^{1.1.}$, $b^{1.1.}$, $c^{1.1.}$, $d^{1.1.}$, of the 16-cell stage (Pl. X, Fig. 11), *all* acquire cilia and constitute the *primary* prototroch (Fig. 18). They are arranged in four groups, which are separated by non-ciliated areas. A little later the cells filling three of these interspaces, the two lateral and the ventral ones, become ciliated, so that a band of cilia surrounds the larva, except in the mid-dorsal line. This completed prototroch consists of twenty-five cells, — sixteen from the upper hemisphere, and nine from the lower. Later in the development the dorsal interruption is obliterated by the concrescence of the prototroch cells from either side, without the addition of other cells. While the interruption still persists, the

undifferentiated ectoderm cells of the upper and lower hemispheres are in free communication at this place, and a few migrate from a position above the prototroch to one below it.

From the 64-cell stage nearly all the cells of the large dorsal quadrant behave very differently from those in the other three quadrants. This is especially noticeable in the descendants of the somatic-plate cells (d^2) and the mesoderm cell (d^4). They do not contribute to the prototroch as do those of the other three quadrants, and they manifest a marked tendency to cleave and arrange themselves bilaterally with respect to the middle line of the embryo.

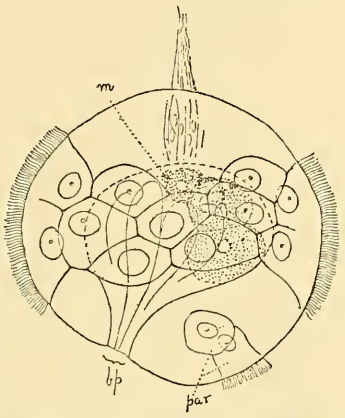

FIG. VI.—Optical sagittal section of trochophore at the time of the closure of the blastopore, with surface view of prototroch and paratroch (par.). Outline of segmentation cavity indicated by dotted line; mesoderm stippled; *m*, minute product of first division of paired mesoderm cells; *bp*, blastopore; the prototrochal cells with nucleoli are from primary, the others from secondary, trochoblasts. From camera sketch.

The paratroch is differentiated at a comparatively early stage and is composed of four cells, descendants of d^2 (Pl. X, Fig. 14), which at first lie in a very slightly curved arc at the posterior or dorsal lip of the blastopore. A few small cells, likewise descendants of d^1 (*X*), also lie within this arc, and, together with the paratrochal cells, mark with absolute certainty the posterior end of the larva (Pl. XIV).

The *mesoblast cell*, d^4, divides once at the surface, and the two resulting cells sink into the segmentation cavity, where they function as teloblasts and give rise to a pair of mesoblast bands. The first division of each, however, occurs during the inward migration, and the spindles lie in the short diameter of the elongate cells, and apparently in the direction of the greatest pressure. The fact that in other forms similar divisions occur at the surface renders this cleavage especially significant (text Figs. V, VI, and IX).

The division of the four cells lying at the vegetative pole (A_4, B_4, C_4, D_4) forms a definitive, cross-shaped, entoderm plate of eleven cells.

The trochophore consists of about two hundred cells when the paratroch is differentiated, while the mesoblast bands, at this time, are made up of four cells each.

III. Formation and Elongation of the Trunk.

a. *Shifting of areas on the lower hemisphere.* — The present chapter will consider cleavage only as a means of orientation in following the movements of embryonic areas, during the metamorphosis of the spherical one-layered trochophore into the elongated three-layered larva.

The prototroch naturally divides the trochophore into an anterior umbrellar and a posterior subumbrellar region. Upon the latter the more important shifting of areas occurs, involving the somatic plate, colored blue ; the mesoderm, red ; the entoderm, stippled; and the rest of the ectoderm, untinted. At a stage when the mesoderm cells are still at the surface (Figs. 38, 40–42), they, together with the entoderm cells, occupy a relatively large area on the subumbrella, even larger than that of the somatic-plate cells.

The mesoderm sinks into the segmentation cavity, and the somatic plate, by spreading out, comes to lie next to the posterior edge of the entoderm plate. In the median line the point of meeting of the two plates is about 90° from the prototroch (text Fig. VII). The eleven entoderm cells begin to invaginate in exactly the same manner, and consequently the surface area of the entoderm plate gradually diminishes until it finally disappears. Since the cells all invaginate at a uniform rate, the pattern of the plate on the surface remains nearly the same in shape, though constantly diminishing in size. The blastopore closes from all directions at once, and the stomodæum is formed where the entoderm cells were last seen on the surface, *i.e.*, about 30° behind the prototroch in the mid-ventral line (later, however, when the œsophagus is formed, the mouth

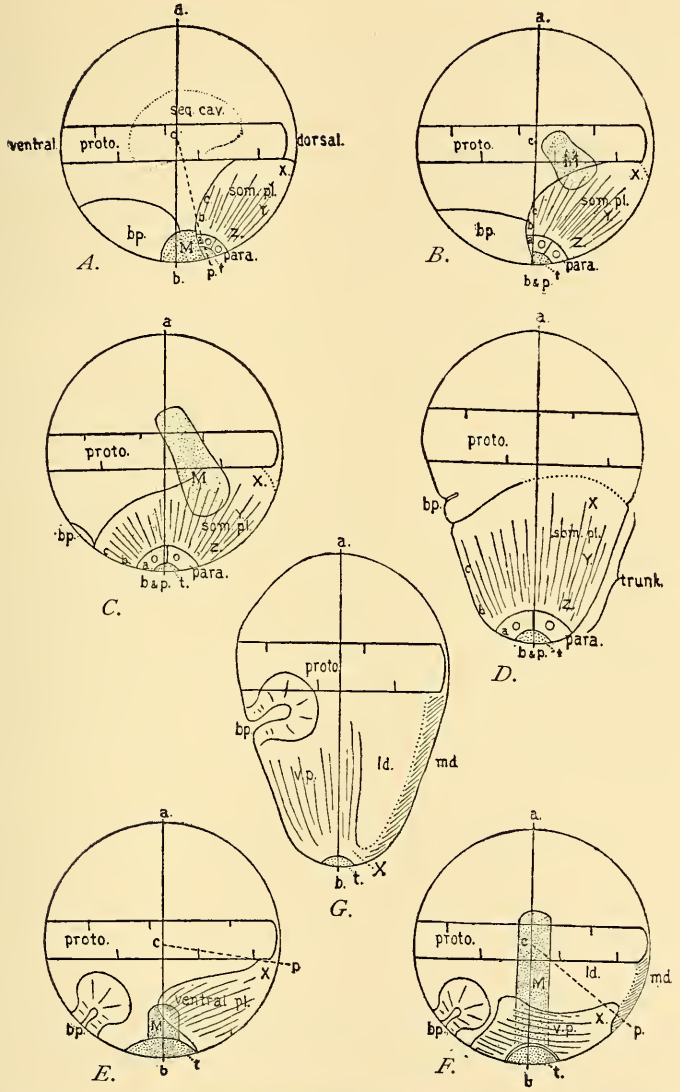

FIG. VII. — Diagrams to express the essential difference in the axial relations and the manner of formation of the trunk between *Nereis* (Wilson [1]), and *Amphitrite* (this paper). *A*, *B*, *C*, and *D*, *Amphitrite*; *E*, *F*, and *G*, *Nereis*. Line *acb*, egg axis; line *acp*, anterior posterior axis; *bp.*, blastopore; *M*, mesoderm; *m.d.*, mid-dorsal region; *l.d.*, latero-dorsal region; *para.*, paratroch; *proto.*, prototroch; *som.pl.*, somatic plate; *t.*, terminal region; *v.p.*, ventral plate; *X*, that part of somatic plate originally nearest the prototroch.

is nearer the prototroch). Thus, the cells which at first occupied a large part of the area of the subumbrella have left the surface entirely and sunk into the cavity. But since the general contour of the egg is but little altered, it is obvious that, meanwhile, other cells must have come to occupy this area. They are those of the somatic plate, and the process in general is simply this: the mesoderm and entoderm constantly sink in and so diminish in surface area; on the other hand, the somatic plate becomes thinner, and extends its surface area until it occupies, not only nearly all its original portion of the subumbrella, but also that of the mesoderm and entoderm.

The manner in which the somatic plate extends into the new area is of especial interest. Upon the invagination of the mesoderm, the posterior border of the somatic plate moves slightly backwards, and meets the entoderm plate at the center of the subumbrella, — the point *b*, text Fig. VII, *A*, *B*. This is the only backward movement which occurs on the border of the somatic plate *in the middle line*. The material at *b* always remains 90° from the prototroch, and finally becomes the posterior end of the metameric larva. The border of the plate on either side continues to move round this pivotal point: its outline is at first convex, but soon becomes nearly straight, then V-shaped, and finally the edges on either side meet and concresce in the mid-ventral region of the embryo (text Figs. VII and VIII).

In this movement the material which shifts its position does not change its *latitude*, *i.e.*, the material of the plate which was at first nearest the prototroch remains always nearest; that nearest the point *p*, or farthest from the prototroch, remains always in this relation, and forms the posterior end of the embryo.

So it comes about that the somatic-plate cells form a cap over the whole posterior portion of the subumbrella. The only other ectoderm cells are in the region of the stomodæum and the few small cells, which, as we have seen, migrated from the upper hemisphere through the interruption in the prototroch. These occupy at most but a limited area just posterior to the prototroch.

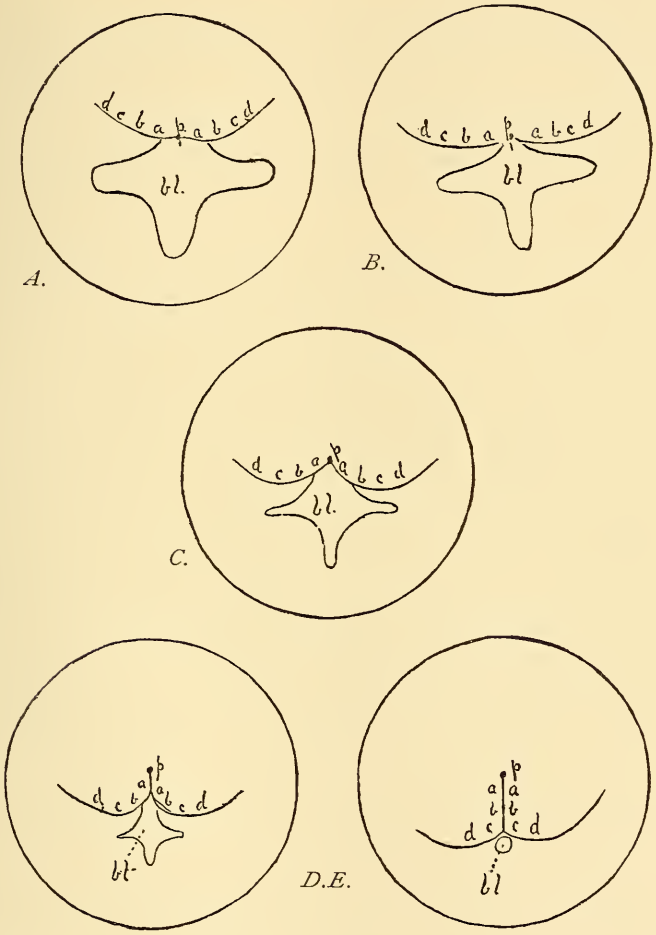

Fig. VIII. — Diagrams of subumbrella showing the lateral extension of the borders of the somatic plate on either side and their final concrescence in the mid-ventral line. The point p is the posterior end; $bl.$, the blastopore; the lines a, b, c, d, the ventral border of the somatic plate. The material at p does not move, but that at a, b, c, d, on either side, moves round as the blastopore closes, and meets that of the other side in the mid-ventral line. As a result the point p is entirely surrounded by cells of the somatic plate.

The figures on Plates III, IV, V show the various stages in the lateral extension of the somatic plate.

Formation of metameres. — By the time the blastopore has closed, the paratroch has become conspicuous, and has nearly attained its ring-like form (Figs. 58, 62). (This is not perfected, however, until after the invagination of the proctodœal cells — about Fig. 64.)

The subumbrella now begins to elongate rapidly owing to the further cleavage of the cells just in front of the paratroch — the budding zone.

The constant parallelism of the prototroch and paratroch indicates clearly the amount of elongation of the trunk region, and shows that it grows on all sides, dorsal, ventral, and lateral, with equal rapidity. The consequent changes of contour are illustrated in the text Figs. X–XVIII. The ectoderm of the trunk becomes thinner and thinner as the latter elongates. The first indication of metamerism is a groove which appears a little behind the prototroch (Fig. 64), and separates the head segment from that of the trunk. I believe that all the ectoderm posterior to this groove arises from the somatic plate, or, in other words, from the descendants of cell d^2 of the 16-cell stage; it is possible that a part of the region in front of the groove is also occupied by somatic-plate cells.

After further elongation, the trunk divides into two segments. The posterior elongates and divides, thus giving rise to three trunk segments. The subsequent elongation of the body is accompanied by the repeated division of the ultimate metamere. In text Fig. XVII, four setigerous segments are outlined, though the prototroch and paratroch still persist at opposite ends of the larva.

No ectodermal teloblasts are distinguishable at any time during the formation and elongation of the trunk.

Although I have not made a complete study of the structures of the late trochophore and the development of the larval organs, I will record the following observations.

Alimentary tract. — The cells of the entoderm plate sink into the segmentation cavity in the manner described above, without producing any marked depression on the surface of the egg (Figs. 61, 62, text Fig. VI). Here they commence to divide and form a solid mass of cells, which, with the mesoderm, completely fills the segmentation cavity.

The ectoderm cells in the vicinity of the blastopore form an œsophagus which acquires a lumen, while the cells of the entoderm are still in a solid mass (Figs. 63, 64). Considerably later the proctodœum is formed from cells within the paratrochal ring which originally lay at the posterior lip of the blastopore, and belonged to the somatic plate (p. 244). About this time the mass of entoderm cells acquires a lumen. The gut is differentiated into a stomach and intestine, and in the walls of the former is found nearly all the yolk.

Fig. XVIII, p. 261, representing a larva eleven days old, shows that the œsophagus, stomach, and intestine are already highly differentiated. The œsophagus is ciliated throughout, and there are numerous strong cilia in the stomach near the narrow cardiac opening.

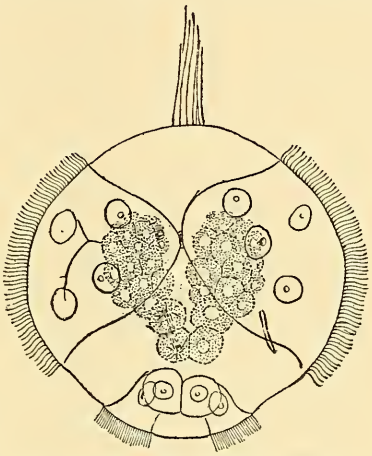

Fig. IX. — Trochophore from the dorsal side, showing prototroch, paratroch, apical tuft, and mesoderm bands within (stippled). From camera sketch.

Mesoderm. — When we last referred to the mesoderm there were four cells in each band (Fig. 61). For some time the number increases by simple division of the teloblasts, and then the single rows are broken by cleavage of the component cells. Even after this, however, one can distinguish the teloblasts lying close together and as near as possible to the posterior end of the embryo (text Fig. IX). In the much elongated larva one can make out in section the mass of undifferentiated mesoderm at the posterior end, and anteriorly the well-defined mesoderm layers lining the gut and body-wall. There is no persistent primary body-cavity.

Mucous glands. — After it is no longer possible to follow exactly the arrangement of the other cells of the upper hemisphere, the dorsal mucous glands *gl.r.* and *gl.l.* can easily be traced, since their nuclei are particularly large and clear and

have peculiar nucleoli (Fig. 59). I have paid less attention to the glands on the ventral side. At a stage like that of Fig. 64 and text Fig. X, the gland cells present a new appearance. The outer end of each cell (the end of each duct), becomes filled with a substance which stains very deeply with methyl-green, hæmatoxylin, etc., and a few hours later this substance fills the duct and the upper part of the body of each of the gland cells (text Figs. XI–XVIII). Meanwhile two other pairs of gland cells appear in the head segment just behind the prototroch. They are bilaterally placed; one pair close together on the ventral side just back of the mouth, the other pair also close together on the dorsal side.

When the larvæ from twenty-four to thirty-five hours old are killed in Perenyi's fluid and stained with Biondi Ehrlich, the numerous glands are brought out in brilliant contrast to the other tissues, for the latter are light red, while the glands are an intense green. The body of each gland becomes greatly distended. In larvæ five days old mucous glands appear also in the paratrochal region. All the glands, at least in the head region, are unicellular and ectodermal.

Nervous system. — The two eye spots which are formed at the beginning of the second day persist until at least the eleventh day, — long after the disappearance of the ciliated organs. They are simple structures, consisting of an outer brown pigment, a clear lens, and apparently an optic nerve.

The cluster of columnar cells immediately under the apical tuft represents, I presume, an apical sense organ (Fig. 64). That the brain arises from the "first group of micromeres," *i.e.*, from the four upper cells of the 8-cell stage, — the "encephaloblasts" of von Wistinghausen,[5] — there can be no doubt; but so do many other organs, for example, the mucous glands, and the greater part of the prototroch. In comparatively early stages the fibrous portion of the brain can be made out directly under the apical pole. I do not know from what cells the brain arises, and, while it may come from the cross cells, as

[5] Wistinghausen, C. v.: Untersuchungen über die Entwicklung von Nereis Dummerillii. *Mitth. a. d. Zool. Stat. zu Neapel.*, Bd. 10, 1891.

Wilson holds for *Nereis*,[1] I see no conclusive evidence, for or against this supposition.

The ventral cord is differentiated as usual from the ectoderm of the ventral plate. Ganglia, connectives, etc., are formed while the larva is still in possession of prototroch and paratroch, and the first ventral ganglion lies in the first setigerous segment.

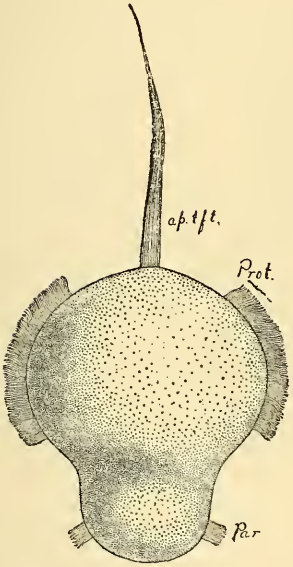

FIG. X. — Trochophore of *Amphitrite*, about 20 hours; *ap.tft.*, apical tuft; *prot.*, prototroch; *par.*, paratroch.

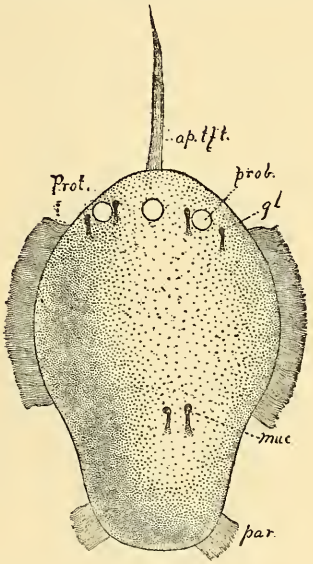

FIG. XI. — *Amphitrite*, 24 hours, ventral aspect; *ap.tft.*, apical tuft; *prob.*, problematic bodies; *gl.*, duct of umbrellar mucous gland; *muc.*, subumbrellar mucous gland; *par.*, paratroch; *prot.*, prototroch.

Problematic bodies. — At about the time the anterior mucous glands begin to react to the methyl-green, there appear in the ectoderm near the openings of the glands five spherical bodies, disposed symmetrically, one on the mid-ventral line, two on the ventral, and two on the dorsal side. They increase rapidly in size up to a certain point, and remain as long as the mucous glands and the prototroch. They appear to be spherical vesicles filled with a fluid, which does not react to methyl-green nor Delafield's hæmatoxylin like the substance of the mucous

glands, but does stain with Zocher's alum-cochineal. I do not understand their structure and function; — possibly they are homologous with the frontal bodies of *Nereis* (Wilson) (text Figs. XII–XVIII).

Cilia. — Cilia are seen first on the sixteen primary cells, and then on all the twenty-five cells of the completed prototroch. Next the paratroch becomes ciliated, and, at about the same time, a tuft appears at the apical pole. The cilia of the prototroch and paratroch at first are very numerous and fine, and, in each case, are distributed in an even zone encircling the

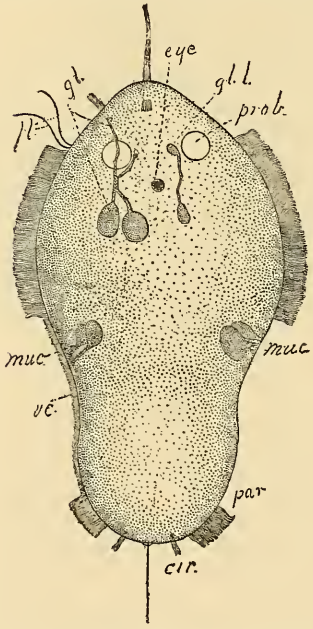

FIG. XII. — *Amphitrite*, 28 hours, left side; *gl.l.*, left dorsal umbrellar mucous gland (opening); *gl.*, ventral umbrellar mucous gland; *fl.*, flagellum; *muc.*, subumbrellar mucous gland; *v.c.*, ventral band of cilia; *cir.*, anal cirrus; *prob.*, problematic bodies.

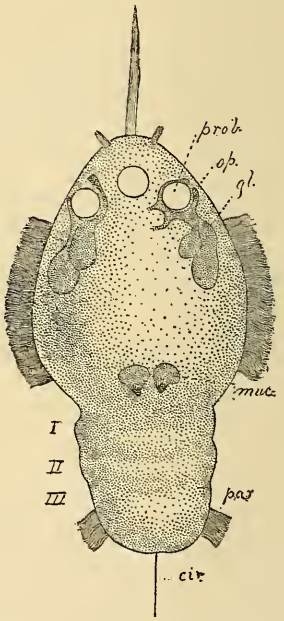

FIG. XIII. — *Amphitrite*, 36 hours, ventral; *prob.*, problematic bodies; *gl.*, ventral umbrellar mucous gland; *op.*, its external opening; *muc.*, subumbrellar mucous gland; *par.*, paratroch; *cir.*, anal cirrus.

trochophore. Later they become much larger, strong, and so tough that they are often fairly well preserved with so harsh a reagent as Perenyi's fluid. The apical tuft which is very large in

young trochophores, atrophies when the body begins to elongate, and a few clumps of short cilia appear upon the umbrellar surface.

Two or three large slowly moving flagella are found in front

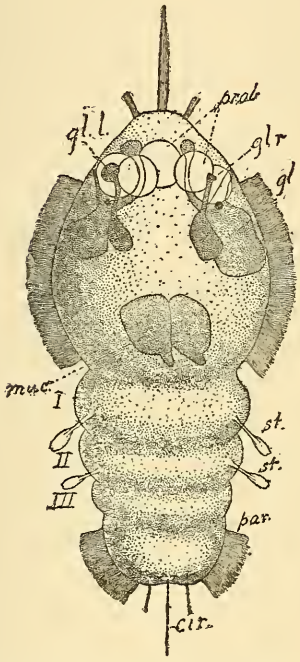

FIG. XIV. — *Amphitrite*, 44 hours, dorsal; *gl.l.*, duct of left dorsal umbrellar mucous gland; *gl.r.*, right dorsal umbrellar mucous gland; *prob.*, problematic bodies, *gl.*, ventral umbrellar mucous gland; *muc.*, ventral subumbrellar mucous gland; *st.*, seta; *par.*, paratroch; *cir.*, anal cirrus; *I*, *II*, *III*, 1st, 2d, and 3d trunk segments.

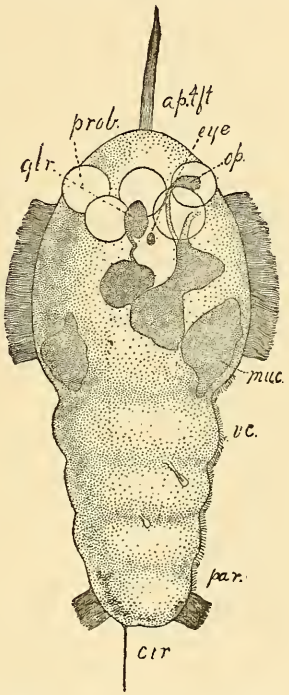

FIG. XV. — *Amphitrite*, 44 hours, right side; *ap.tft.*, apical tuft; *gl.*, and *op.gl.*, ventral umbrellar mucous gland and its external opening; *gl.r.*, right dorsal umbrellar mucous gland; *prob.*, problematic bodies; *muc.*, subumbrellar mucous gland; *v.c.*, ventral band of cilia; *par.*, paratroch; *cir.*, anal cirrus.

of the prototroch in the mid-ventral line. A ventral band of short cilia connects the prototroch and paratroch, and a few ciliary tufts and a large cirrus occur in the region behind the paratroch (text Figs. X–XVIII).

Setæ, parapodia, etc. — When the larva has elongated and has begun to divide up into metameres, it develops in each

segment a pair of oar-shaped setæ, and soon after one or more pairs which are needle-shaped, while the body-wall is evaginated to form parapodia, in which seta-muscles, etc., are developed. Shortly after the dorsal setæ are developed, there appear in all the trunk segments, except the first, some little hooks, the setæ of the ventral parapodia (text Figs. XII–XVIII).

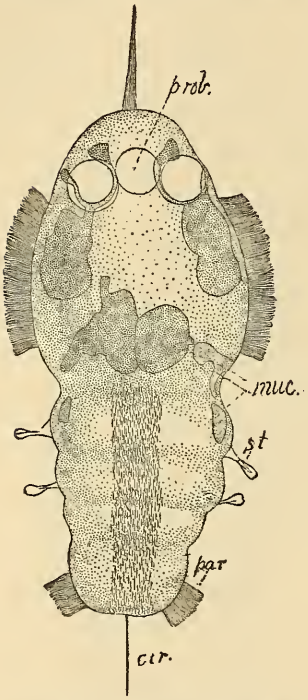

FIG. XVI. — *Amphitrite*, 44 hours, ventral aspect; *prob.*, problematic bodies; *gl.* mucous gland; *muc.*, subumbrellar mucous gland; *st.*, seta; *par.*, paratroch; *cir.*, cirrus.

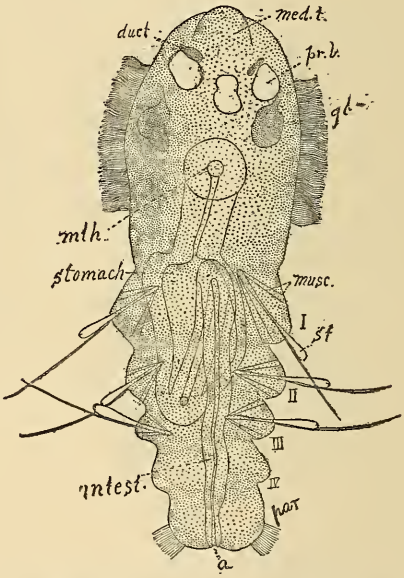

FIG. XVII. — *Amphitrite*, ventral aspect, 60 hours; *med.t.*, median tentacle; *pr.b.*, problematic body; *gl.*, mucous gland; *mth.*, mouth; *musc.*, setæ-muscles; *st.*, setæ; *par.*, paratroch; *a.*, anus; *I, II, III, IV*, trunk segments.

IV. METAMORPHOSIS FROM FREE-SWIMMING TO CREEPING LARVÆ.

When the larvæ have developed about five trunk segments, they no longer swim about freely, but sink to the bottom and become adapted to a less active mode of life, and if delicate pieces of fresh ulva are put into the aquarium, they thrive much better.

The prototroch and paratroch, before their actual disappearance, undergo a marked degeneration. The cells shrink and become filled with yellow granules, and within a comparatively short time all the ciliated tracts on the surface and all the mucous glands disappear. These phenomena, together with the fact that the glands occur in the region of the prototroch and paratroch, indicate that they are correlated physiologically with the cilia. The problematic bodies also partially collapse and then disappear, and a *median tentacle* is formed by an evagination of the body-cavity just ventral to the apical pole. Since this appears before the ducts of the mucous glands and the problematic bodies have entirely degenerated, it is a valuable means of orientation in later stages (text Figs. XVII, XVIII).

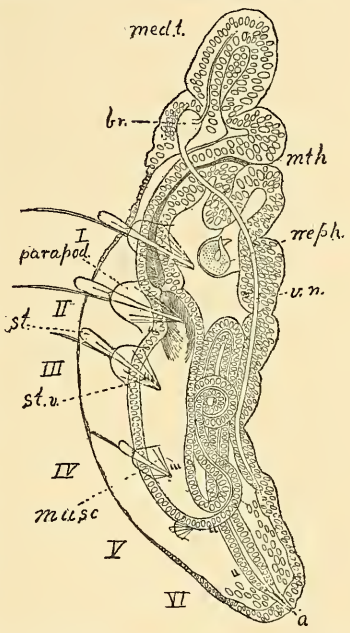

FIG. XVIII. — *Amphitrite*, 11 days, optical section: *med.t.*, median tentacle; *br.*, brain; *mth.*, mouth; *neph.*, nephridium; *v.n.*, ventral nerve cord; *parapod.*, parapodia; *st.*, seta (dorsal); *st.v.*, setæ (ventral hooked); *musc.*, muscles of setæ; *I*, *II*, *III*, etc., trunk segments.

Text Fig. XVIII shows the large mouth, the position of brain, cord, eyes, nephridia, etc.

B. CLYMENELLA TORQUATA VERRILL.

Habits and cleavage. — The breeding habits of *Clymenella torquata* in the vicinity of Woods Holl are very different from those of *Amphitrite*. While the latter may be found breeding at any time during the summer, but never in great abundance, the breeding season of *Clymenella* lasts but two or three days, varying in different years from the last of April to the middle of May, and almost every normal individual discharges its sexual products at this time. The worms which are found

nearly everywhere in light sand or gravel just below low-water mark, live in slender sand-tubes.

Under the most favorable circumstances I have sought in vain for the eggs deposited under natural conditions, and believe they are discharged free into the water, usually at night, and scattered by the tides.

Since one is liable to be prevented by the high winds prevalent in May from getting the worms at just the right time, the surest way to secure the eggs is to collect a large number of worms just a few days before the breeding season. The males and females can be distinguished, since the eggs show plainly through the body-wall. If the females are kept in an aquarium without sand, the eggs will never be laid, though the worms may live for weeks; nor can the eggs be fertilized, under these circumstances, if cut out of the body-cavity. If, however, an abundance of sand be furnished, they build new sand-tubes, and deposit their eggs on the surface of the sand at the mouth of the tube.*

E. B. Wilson describes the *Clymenella*, which occurs in great numbers at Beaufort, N. C., as laying its eggs in jelly masses the size of pigeon's eggs. These masses are attached to one of the two openings of a Y-shaped tube, and are found all summer in great abundance. In the Woods Holl form many of the Y-shaped tubes are found in the breeding season, but there are no jelly masses. The wide divergence in the breeding habits of the northern and southern forms is remarkable, though it is possible that the worm which Wilson describes is *Axiothea mucosa*, and is generically different from the northern *Clymenella torquata* (cf. Andrews [6]).

The unfertilized egg is practically spherical, about 150μ in diameter, and perfectly opaque, being filled with a large amount of yellow yolk. They may remain unfertilized in the sea-water for several hours at least and still be capable of developing normally.

* If finely broken glass be substituted for sand, they will use this in the construction of their tubes.

[6] Andrews, E. A.: Report upon the Annelida Polychæta of Beaufort, N. C. *Proc. U. S. Nat. Mus.*, vol. XIV, p. 277.

The egg is oriented in precisely the same way as is *Amphitrite*. Up to the time when the sperm enters the egg, the first maturation spindle is in the equatorial-plate stage, and the polar globules remain to mark the apical (animal) pole. The first furrow depresses the surface first at the animal pole, as in *Nereis*.

A detailed account of the later cleavage processes in *Clymenella* would be little else than a repetition of the above description of *Amphitrite*. *Every division up to the 64-cell stage takes place in the same direction in both species.* There is, moreover, a most striking resemblance in the relative size of the cells, though the differences demand attention: the cell D_2 is larger in proportion than in *Amphitrite;* the four upper cells of the 8-cell stage, and therefore the whole anterior hemisphere of *Clymenella* is relatively smaller — among themselves, however, the cells in this hemisphere have about the same relative size as in *Amphitrite;* the mesoderm cell *M* is comparatively large; the sixteen descendants of the trochoblasts (16-cell stage) have the same arrangement, and *all* become prototrochal cells in the same manner; the apical rosette and cross arise in the same way; the seven cells, which at the 64-cell stage constitute the entoderm plate, are in the same position; the relative size of $a^{2.2}$, $b^{2.2}$, $c^{2.2}$, and $x^{1.2}$ ($d^{2.1.2}$) is the same. x^2 (= $d^{2.1.2}$), however, is somewhat smaller, and this difference may be of considerable theoretical importance in connection with the formation of the paratroch, since three-fourths of the paratroch in *Amphitrite* is formed from this cell (Pl. XV).

Remarkable as are the similarities between the early cleavage stages in these two worms representing rather distantly related families (Maldanidæ and Terebellidæ), some of the subsequent phenomena show even more striking resemblances. The apical rosette cells divide in the same manner (Figs. 71, 72). Each of the "secondary trochoblasts," $a^{2.1}$, $b^{2.1}$, and $c^{2.1}$ divides into a group of four cells: three larger and subequal, and the fourth minute. These groups correspond in the three quadrants. *The three larger cells in each group become ciliated and complete the prototroch, while the minute one does not enter it. Thus, the whole prototroch in Clymenella arises in precisely*

the same way and from the very same cells as in Amphitrite (Figs. 80, 81, 83, 85, 86, text Fig. VI).

The lowest cells A_4, B_4, C_4, D_4 (64-cell stage) divide once more on the surface as in *Amphitrite*, resulting in a definitive entoderm plate of eleven cells. The position of these, however, is slightly different: a^4 and a^5 border upon A_5 in *Clymenella*, while only a^5 does so in *Amphitrite*. The same arrangement obtains in the quadrants *b* and *c*. A_5, B_5, and C_5 each develops a large "vacuole," which can be followed for a long time after the invagination.

Figs. 78–88 show that the divisions of the somatic-plate cells, and those of the other ectoderm cells, $a^{2.2}$, $b^{2.2}$, $c^{2.2}$, and a^3, b^3, c^3, as far as they have been followed, agree closely in the two forms. The paired mesoderm cells *M* and *M* divide *about equally upon the surface*, while in *Amphitrite* this division is very unequal (p. 248).*

The trochophores of the two annelids have a general resemblance, though *Clymenella* is less active.

Summary. — Though the egg of *Clymenella* is much larger than that of *Amphitrite*, and though the worms belong to entirely different families, the similarity in the cleavage is very remarkable.

Up to the 64-cell stage all the cleavages correspond in direction, and within certain limits the relative size of the cells is the same. In the later stages, there is still a wonderful resemblance even in details; the formation of the rosette, cross, and prototroch, and the division of the somatic plate up to eleven cells at least is identical. The entoderm (?) and the mesoderm (?) is derived from the same cells in both forms.

The axial relationships as far as followed are the same in both annelids.

Clymenella differs from *Amphitrite* in the absolute size of the egg, in the relative size of the anterior and posterior hemispheres, the larger size of d^4 (mesoderm cell), the earlier division of *M* and *M on the surface*, the relative size of the products of this division, and the smaller size of X^2.

* Of course in calling these cells *entoderm* and *mesoderm*, I am presuming that their destiny as well as their origin is like that of other forms.

C. LEPIDONOTUS SP.

Habits and cleavage. — The breeding season of *Lepidonotus* at Woods Holl extends from the last of April nearly to the first of June.* The adult worms are commonly found under stones and mussel-beds, and are easily captured, for, when disturbed, they do not attempt to escape, but roll up like porcupines, and depend on their tough dorsal scales for protection. There is no difficulty in separating the males and females, for the former are whitish while the latter are dark on the ventral surface.

It seems that the females may carry the fully ripe eggs for a long time before laying them, for nearly all those captured, even *early* in the breeding season, can be induced to deposit their eggs the day they are collected.

In captivity the worms will rarely lay in the daytime. At night, more frequently from 8 to 10 o'clock, they can usually be persuaded to discharge their eggs, if they are suddenly plunged into colder water, and held up close to the lamp.

The animals remain at the bottom of the dish, and, except for a slight tremor, do not move, while the eggs or sperm stream in delicate threads from the eighteen pairs of nephridial pores. As in the two species described above, the eggs may be kept in sea-water several hours before fecundation. I have fertilized eggs at 8 o'clock in the morning which were laid some time during the night by isolated females, and once purposely kept eggs in sea-water from 5 until 8.30 P.M. before fertilizing them. They developed perfectly well in both cases.

When first laid, the eggs are quite opaque, and very irregular in form. They soon become approximately spherical and about 65μ in diameter. The rapidity of development depends directly upon the temperature of the water (see further on p. 269): at 8° C. the eggs reach the 2-cell stage in about 2½ hours after fertilization; the first cleavage furrow is completed in five minutes and sinks in all round the eggs at the same time, and not first at the animal pole, as in many eggs with abundant yolk like *Nereis* and *Clymenella*. Fig. 89 illustrates the point.

* They may breed earlier.

It represents four superimposed profile views of the egg during the first cleavage, drawn at intervals of about one minute.

The division of the first two blastomeres is equal, simultaneous, and slightly oblique, so we have a 4-cell stage in which the quadrants cannot be distinguished from one another. The cross-furrows at the vegetative and animal poles are about equal in length, and at right angles to each other, so that, as in *Amphitrite*, two of the diagonally opposite cells are higher than the other two. These four cells — the anlagen of the four quadrants — divide simultaneously into eight cells by a right-oblique cleavage (Figs. 90, 91), those of the anterior hemisphere being slightly smaller than the other four. The next division is synchronous and left oblique, and the resulting sixteen cells are nearly equal in size. They soon divide obliquely to the right, but not all exactly at the same time, though the corresponding cells of the four quadrants, or, as Kofoid tersely expresses it, "the cells of the same quartette," divide simultaneously: the upper quartette $a_{1\cdot1}$, $b_{1\cdot1}$, $c_{1\cdot1}$, $d_{1\cdot1}$, and that at the vegetative pole, A_2, B_2, C_2, D_2, divide first; next the trochoblasts $a^{1.1}$, $b^{1.1}$, $c^{1.1}$, $d^{1.1}$; and shortly after $a^{2\cdot}$, $b^{2\cdot}$, $c^{2\cdot}$, $d^{2\cdot}$. In Fig. 92 the first two quartettes have already divided, and the two last are dividing. When these divisions are completed, the egg is in a typical 32-cell stage, and the cells are of about the same size, except the four larger at each pole.

While the egg remains in the 32-cell stage the polar globules, which are still attached to the egg at the animal pole, usually penetrate into the cells $a_{1\cdot2}$, $b_{1\cdot2}$, $c_{1\cdot2}$, or $d_{1\cdot2}$. They may enter the same, adjacent, or even opposite cells, and rarely one works its way between the cells into the segmentation cavity. Curiously enough this phenomenon always takes place during the 32-cell stage.

The thirty-two cells all divide obliquely to the left, and the corresponding cells in each quadrant divide synchronously. The sequence of the quartettes is the same as in the previous generation; but in case of sister cells the larger divides first. To be more specific, the first cells to divide are $a_{1\cdot2}$, $b_{1\cdot2}$, $c_{1\cdot2}$, $d_{1\cdot2}$ and A_3, B_3, C_3, D_3; then, $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, $d^{1.2}$ and a^3, b^3, c^3, d^3; $a^{1.1.2}$, $b^{1.1.2}$, $c^{1.1.2}$, $d^{1.1.2}$; $a^{2.1}$, $b^{2.1}$, $c^{2.1}$, $d^{2.1}$; $a^{2.2}$, $b^{2.2}$, $c^{2.2}$, $d^{2.2}$ (cf. Figs. 93–97).

The result of these divisions is a 64-cell stage, which is like that of *Amphitrite* and *Clymenella*, except that it is more regular; so regular, in fact, that there is no way of distinguishing one quadrant from another. It is easy to distinguish the two poles and thus the egg axis, but not the sagittal plane of the embryo. One can distinguish from one another the component cells of a quadrant, but cannot say to which quadrant they belong. This is true throughout the history of the cleavage, as far as I have studied it.

The apical rosette is formed just as in *Amphitrite*, *Clymenella*, etc., though the origin of the cells is peculiar in that they lie at first in the segmentation cavity but later elongate and reach the surface (Fig. 98). Since the polar globules may be in any of the cross cells, they are sometimes found far removed from their original position (p. 9, Figs. 98, 100, 101, 104).

At the vegetative pole the eight lower cells, A_4, B_4, C_4, D_4 and a^4, b^4, c^4, d^4, form what may be called provisionally the entoderm plate. These do not divide again at the surface, but become elongated, and their nuclei migrate inward like those of the mesoderm and entoderm cells of *Amphitrite* (Figs. 102, 103).

It is interesting to notice the contrast between the divisions of the animal and the vegetative-pole cells in this last cleavage. The two cell-groups in question were of about the same size and similarly arranged, four cells at the animal pole and four at the vegetative pole. All the cells of both groups divide in the same direction and at the same time, but at the animal pole the result is eight cells, of which the four central ones are the smaller, while at the vegetative pole the result is exactly the reverse — the four central cells are the larger. The relative position of the cells at the two poles of the egg can be seen in Fig. 95.

At the 64-cell stage the living egg shows distinctly the rosette, undivided cross cells, and the prototrochal cilia borne, in part at least, by the cells corresponding to the primary prototroch in *Amphitrite* (Fig. 97, shaded cells).

The situation of the fully formed prototroch and the size and position of its component cells sustain the interpretation

that the primary prototroch is the same as in *Amphitrite* and *Clymenella*. Figs. 100, 101 show that the further divisions of the cross cells correspond in direction exactly to those in *Amphitrite*, while other ectoderm cells conform to the law of alternating oblique cleavage.

I have been unable to discover the origin of the mesoderm in *Lepidonotus;* it may come from d^4 as in other forms, but d^4 cannot be distinguished from a^4, b^4, and c^4, and all of these cells invaginate together with A_4, B_4, C_4, D_4. In the typical gastrula (Fig. 104), between the entoderm and ectoderm, cells are sometimes found which are apparently mesoblastic in origin. During the gastrula stage the trochophore assumes a remarkable form, seen in text Fig. XIX. The membrane stands out from the body except at the regions of the prototroch and apical tuft. A much later larva is shown in text Fig. XX — a form maintained for several days.

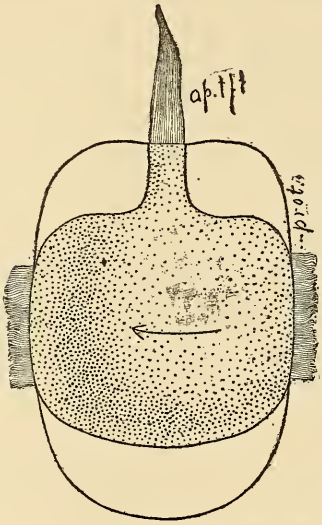

FIG. XIX. — *Lepidonotus*, very young trochophore; *ap.tft.*, apical tuft of cilia; *prot.*, prototroch; the arrow indicates the direction of rotation.

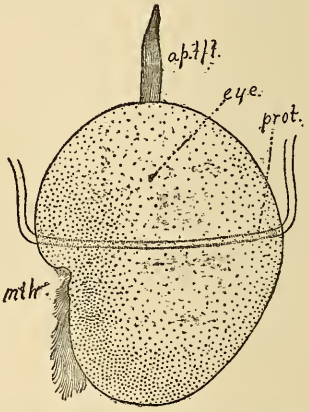

FIG. XX. — *Lepidonotus*, left side, second day; *ap.tft.*, apical tuft; *prot.*, prototroch; *mth.*, mouth.

Influence of temperature, direct heat, and light. — The influence of the temperature of the water upon the rapidity of the cleavage processes is very pronounced. The difference of

a few degrees between the first and the last of the breeding season appreciably increases the rate of development. If artificial heat is applied, even a sudden rise of many degrees does not have the slightest effect on the manner of development, but greatly increases the rapidity. Of several hundred eggs fertilized at 8 A.M. on May 1, the larger number were in the 2-cell stage at 11.30 A.M., though some of them were dividing into four. The eggs were then separated into two dishes. The temperature in dish *A* was kept at that of the sea, 8° C. The other dish, *B*, was placed in a warm bath, and at 11.40 the temperature had risen to 35° C. It rapidly fell to 21°, and there remained. In dish *A* the development continued very slowly. In dish *B*, where the temperature was raised 17° in ten minutes, the eggs took a sudden start and developed twice as quickly as those in the cold water.

	Dish *A*, 8° C.	Dish *B*, 21° C.
11.40 A.M.	2– 4 cells,	2– 4 cells.
12.07 P.M.	2– 4 "	4– 8 "
1.05 "	4– 8 "	16–32 "
3.35 "	8–16 "	32–64 "
4.00 "	16+ "	64+ " (swimming).
4.10 "	—— "	swimming rapidly.

The reaction of larvæ to light varies with age. When they first begin to swim, they are positively heliotropic, but later, when they are about fourteen hours old (text Fig. XX), most of them are negatively heliotropic. From twenty to about forty hours after fertilization they are apparently indifferent to light, and are scattered evenly throughout the water. At about forty-four hours they exhibit a very marked positive heliotropism, which continues for several days at least.

Larvæ three days old which were strongly heliotropic, crowding to the sides of the dish nearest the window, could not be induced to abandon this position by direct heat rays acting opposite to, at right angles to, or coincident with, the light rays.

The eggs of *Harmothoë sp.* are slightly smaller and clearer than those of *Lepidonotus*, and develop in almost exactly the same manner. I have tried to obtain hybrids between these species, but without success.

D. SCOLECOLEPIS VIRIDIS VERRILL.

The breeding season of this species is nearly over by the first of May. The eggs are deposited inside the sand-tubes in which the females live. They are of medium size, and of bright yellow color. The membrane does not adhere so closely as in some other species (Fig. 105). The first division is unequal. At the next division, the smaller cell very often divides first. In the 4-cell stage *D* has a relative size even larger than usual. The four cells divide almost synchronously into eight, in the usual right-oblique direction. The peculiarity of the 8-cell stage is that the apical cells, a_1, b_1, c_1, d_1, are very small and perfectly transparent, while the vegetative cells are very large and perfectly opaque.

The four lower cells divide again, and the lower products of this division, A_2, B_2, C_2, D_2, are all opaque. Of the four upper products, a^2, b^2, and c^2 are transparent and comparatively small, while d^2 (somatoblast in other forms) is opaque and of enormous size. Very soon the four apical cells, a^1, b^1, c^1, d^1, divide in the usual left-oblique direction (Figs. 110–113).

Though all the previous divisions and the present positions of the cells are the same as in *Lepidonotus* at the 16-cell stage, the relative size of the blastomeres presents a remarkable contrast (Fig. 112). The cells colored brown in Figs. 112 and 114 correspond in origin to the primary trochoblasts of other forms, — *Amphitrite*, *Clymenella*, and *Nereis*.

Notwithstanding the great differences in the size and in the constitution of these sixteen cells, they all divide again almost simultaneously, with the exception of the trochoblasts $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, $d^{1.2}$, the direction alternating as usual with that of the previous division. The failure of the primary trochoblasts to divide with the other cells prevents the egg from actually attaining the typical 32-cell stage. The difference between the relative size of the cells of the *d* quadrant and that of the corre-

sponding cells of the other quadrants is greater in *Scolecolepis* than in any other form I have studied, Fig. 114 (shaded nuclei). For example, $d^{2.1}$ is the largest cell in the egg, $a^{2.1}$, $b^{2.1}$, $c^{2.1}$ are the smallest even more minute than the primary trochoblasts. They correspond in origin to the *secondary trochoblasts in Amphitrite and Clymenella.* This diminutive size of the primary and secondary trochoblasts is significant in view of the suppression of the trochophore in this form.

Owing to the lack of material, I made only the following observations: an apical rosette is formed in the ordinary manner; a typical gastrula stage with elongated blastopore is present somewhat later; the elongated trochophore has a weak prototroch and paratroch; the head segment is composed of clear cells, and contains four unicellular mucous glands lying in a row in front of the mouth, while the body segments are filled with yolk and covered with a layer of epithelial cells (Figs. 115, 116).

E. CHÆTOPTERUS PERGAMENTACEUS CUVIER.*

The eggs of this rare annelid were obtained in August, 1894, by cutting open the females, and were artificially fertilized with sperm obtained from the male in the same manner.

The egg, which is about equal in size to that of *Lepidonotus*, remains with the first maturation spindle in the equatorial-plate stage, until the entrance of the sperm, as in *Clymenella.* The male pronucleus reaches a position near the center of the egg without following any constant path, and there awaits the female pronucleus. The latter moves toward it along the radius of the egg which terminates at the polar globules. Before the pronuclei unite, the two centrosomes derived from the sperm center are already far apart, one on either side of the male pronucleus. Their position indicates the direction of the first cleavage spindle, since a line connecting the two centrosomes coincides with the axis of the spindle. This is perpendicular to the copulation path of the pronuclei, *i.e.*, the egg axis. It follows that the horizontal plane in which the cleavage spindle will lie

* For maturation and fecundation, see Mead.[24]

can be predicted at least as soon as the first maturation spindle appears, and before the sperm enters the egg.

It is clear that the determining factor is neither the path of the spermatozoön, for the relation of the latter to the direction of the spindle varies; nor the path of the female pronucleus in approaching the male pronucleus, for the two male centers have already assumed their permanent position.

The first furrow is meridional as usual, and sinks in all around the egg at the same time, as in *Lepidonotus*. During the first cleavage a peculiar lobe is normally formed on the lower hemisphere, first becoming noticeable when the spindle is in the equatorial-plate stage. It is composed very largely of yolk, although protoplasmic rays from the astrospheres run through it to the periphery. Every phase in the development of this lobe bears a constant relation to that of the karyokinetic spindle (Fig. 117, 118, etc.). During the later phases of the karyokinesis, — the reconstitution of the nuclei, — the lobe becomes constricted at its base, and finally, by the time the new nuclei have assumed a spherical form, is completely resorbed.*

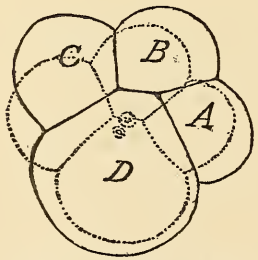

Fig. XXI. — *Chætopterus*, 8-cell stage from lower pole.

The cell *CD*, which bears the lobe, is larger than *AB*. In these two cells the whole karyokinetic process from first to last is carried on simultaneously. The spindles in the two cells are inclined to each other, as in *Amphitrite* (left-oblique cleavage).

The four resulting cells divide simultaneously into eight, in the usual direction. Of the 8-cell stage, the four vegetative cells are, as usual, larger than the four animal cells, and one cell, DD_1, is largest of all (text Fig. XXI). The 8-cell stage of this form is peculiar, however, in that one of the four apical cells, *d*, is much larger than the other three. There are in this stage two

* I said in my previous paper that the lobe was completely constricted off. The statement was based on the study of preserved material which certainly supported this interpretation; but an examination of living eggs plainly shows that I was in error

cross-furrows, one at the animal and one at the vegetative pole, which, instead of being at right angles to each other, are parallel. The eight cells divide in the usual direction and form a 16-cell stage (Figs. 122, 123), in which one of the apical cells $d_{1.1}$, is remarkably large, while its sister cell, $d^{1.1}$, trochoblast, is no larger than $a^{1.1}$, $b^{1.1}$, or $c^{1.1}$. In other respects the 16-cell stage is like that of *Amphitrite* (cf. Figs. 8–122, 11–123).

The nearly synchronous cleavage of all these cells gives us the 32-cell stage (Fig. 124). Every cell is located as in *Amphitrite*, and their relative size is the same with two exceptions: $d^{1.2}$ receives the extra material bequeathed by the cell d_1 of the 8-cell stage, and the four apical cells are of about the same size, — in *Amphitrite* the two dorsal ones are the larger.

Every one of the thirty-two cells divides obliquely to the left, but not synchronously. Already (Fig. 124) three of the cells have spindles, while the others are in the so-called resting stage. The familiar rosette is formed in the typical fashion, and most of the other divisions take place as indicated in the remaining figures by spindles or arrows (Figs. 124–130). On account of the regularity of the cleavage and its resemblance to that of *Amphitrite*, I thought it unnecessary to introduce more figures.

The division of the primary trochoblasts, colored brown, may be seen in Figs. 125–128; the position of the secondary trochoblasts and of $c^{2.1.1.}$ and $c^{2.1.2.}$ in Fig. 132; the mesoderm cells, the above-mentioned rosette, and the primary cross-cells in Figs. 126–131.

An actual 64-cell stage does not occur in *Chætopterus*, owing to the precocious division of certain cells. Soon after the rosette cells are formed, they ingest the polar globules just as do the apical cells of *Lepidonotus* at an earlier period (Figs. 127, 131, 132, p. 266).

I have carried the cell lineage little beyond what may be called the ideal 64-cell stage, but some of the next divisions are of great interest. The primitive cross-cells (colored blue in figures), although in origin and in position exactly like those in *Amphitrite*, divide strictly according to the rule of alternating cleavage, and do not form the cross (Fig. 131). They usually

divide bilaterally (cf. *Amphitrite*, *Clymenella*, *Nereis*, etc.), without exhibiting a trace of the alternating oblique cleavage. Other anomalous divisions occur in the cells corresponding to the primary prototroch of *Amphitrite* and *Clymenella*. Figs. 131, 132 show the cleavage of one of these cells, and I have seen others divide.

Wilson[7] described the larva of this form as having no prototroch. See also Korschelt and Heider.[8] It is possible that there is some correlation between the anomalous behavior of the trochoblasts and the absence of the prototroch.

[7] E. B. Wilson: Observations on the Early Developmental Stages of Some Polychætous Annelids. *Stud. Biol. Lab. Johns Hopkins Univ.*, vol. II, 1882.

[8] Korschelt und Heider: Lehrbuch der Entwicklungsgeschichte.

PART II. — COMPARATIVE AND GENERAL.

In the literature on the embryology of annelids, the record of cell lineage has usually been incidental to the description of the general development of the embryo. Whitman, however, in his well-known work on the embryology of *Clepsine*, 1878, gave a detailed account of the exact origin of those cells which give rise to the mesoderm, nerve-cords, nephridia, etc.; he introduced and proved the value of cell lineage as a method of embryological research.

By far the most important contribution to the cell lineage of annelids since 1878 is that of E. B. Wilson, based on a study of the marine annelid *Nereis limbata*, 1891. He recorded every cell division up to the 58-cell stage, and many of the subsequent cleavages. In the appendix to his paper he briefly describes the cleavage of *Polymnia*, *Spio*, *Aricia*, and *Eupomatus*. Several investigators have shown that the cleavage in certain flatworms and molluscs has many features in common with that in annelids ; the most apparent distinguishing peculiarities are (a) the obliquity of all the early cleavage furrows, and (b) their regular alternation in direction in successive generations of cells. Cleavage characterized by these features may be designated *alternating oblique* (spiral type of Lang, Wilson, and others), and has already been described in the following forms.

Among annelids :

Clepsine, Whitman [9], [10]
Nereis cultrifera, Salensky [11]
Nereis Dummerillii (free-swimming trochophore), Goette [12]
Lumbricus and *Rhynchelmis*, Vejdovsky [13]

[9] Whitman, C. O.: The Embryology of Clepsine. *Quart. Jour. Micr. Sci.*, XVIII, 1878.

[10] Whitman, C. O.: The Germ-Layers in Clepsine. *Journ. of Morph.*, vol. I, 1887.

[11] Salensky, W.: Études sur le Développement des Annélides. *Arch. de Biologie*, III, 1882.

[12] Goette, A.: Abhandlungen zur Entwicklungsgeschichte der Tiere. Leipzig, 1882.

[13] Vejdovsky, Fr.: Entwickelungsgeschichtliche Untersuchungen. Prag, 1883.

Eupomatus uncinatus, Hatschek [14]
Nereis Dummerillii (suppressed trochophore), von Wistinghausen [5]
Nereis limbata and *megalops*, Wilson [1]
Hydroides sp., "
Polymnia nebulosa, "
Spio fulginosus, "
Aricia fœtida, "
Amphitrite ornata, Mead [25] *
Clymenella torquata, " *
Scolecolepis viridis, " *
Lepidonotus sp., " *
Chætopterus pergamentaceus, Mead.[24] *

Among flatworms :

Discocœlis, Lang.[15]

Among molluscs :

Planorbis, Rabl [16]
Neritina, Blochmann [17]
Crepidula, Conklin [18, 19]
Umbrella, Heymons [2]
Unio, Lillie [3]
Limax, Kofoid [4]
Physa, Crampton [20]
Limnœa, "
Patella, Patten.[21]

[14] Hatschek, B.: Entwicklung der Trochophora von Eupomatus uncinatus Phillippi. *Arb. zool. Inst. Univ. Wien*, Bd. 6.

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[16] Rabl, C.: Ueber die Entwicklung der Tellerschnecke. *Morph. Jahrb.*, vol. V, 1879.

[17] Blochmann: Ueber die Entwicklung von Neritina fluviatilis. *Zeitschr. f. wiss. Zool.*, Bd. 36, 1882.

[18] Conklin, E. G.: Preliminary Note on the Embryology of Crepidula fornicata and Urosalpinx cinerea. *Johns Hopkins Univ. Circ.*, vol. X, no. 88, 1891.

[19] Conklin, E. G.: The Cleavage of the Ovum in Crepidula fornicata. *Zool. Anz.*, Jahrg. 15, 1892.

[20] Crampton, H. E.: Reversal of Cleavage in a Sinistral Gasteropod. *Annals N. Y. Acad. Sci.*, vol. VIII, 1894.

[21] Patten, W.: The Embryology of Patella. *Arb. zool. Inst. Univ. Wien*, Bd. 6.

[24] Mead, A. D.: Some Observations on the Maturation and Fecundation of Chætopterus pergamentaceus Cuvier. *Journ. of Morph.*, vol. X, no. 1.

[25] Mead, A. D.: Preliminary Account of the Cell Lineage of Amphitrite and other Annelids. *Journ. of Morph.*, vol. IX, no. 3.

* Previous chapters.

The following chapters are devoted to a comparison of these examples of the alternating type of cleavage from the point of view of (1) *homology of cells*, (2) *developmental mechanics*, and (3) *axial relationships of the embryos*.

I. Homology of Cleavage Cells.

The importance of the similarity in the cleavage of certain annelids, molluscs, etc., has not been unduly emphasized even by those who have worked on these forms, and has been decidedly underestimated by those who have based their interpretation of cleavage upon the echinoderm and the vertebrate egg. Since my own observations tend to enhance the significance of these similarities, I have been led to make the homology of cells a standpoint for comparison.

Essential similarity in origin and fate will be considered a sufficient criterion of the homology of cells, as it is, by common consent, of tissues and organs. That the cleavage cells in the forms under consideration are similar in origin is obvious, and is implied by the fact that they constitute a well-defined type of cleavage; but whether they are alike in destiny is more difficult to ascertain.

The products of the two divisions of the oöcytes — the polar globules — correspond in origin and destiny. The cleavage proper may be "equal" or "unequal."

a. *Equal cleavage.*

Hatschek followed the equal cleavage of *Eupomatus* in detail to sixteen cells, and Wilson describes that of the related *Hydroides* to thirty cells. The observations on *Lepidonotus* recorded in the previous chapters agree precisely with these, and have been extended to the later cleavage.

The first cleavage furrow is always vertical, but in unequal cleavage the two resulting cells vary more or less in size. The larger size of one product is a means of orientation, and since in all cases it possesses certain potential peculiarities, it may be considered in a general way homologous

among eggs of this type. Is the larger size of the cell the cause of its peculiar destiny (according to the law of progressive differentiation), or is the peculiar destiny the cause of its larger size (precocious segregation of embryonic material and specific cell organization)? The answer to this question depends upon *whether one of the two cells in equal cleavage is homologous with the larger cell in unequal cleavage.* This is the immediate problem to be solved by the study of equal cleavage.

In this type the two blastomeres divide into four cells of equal size, the anlagen of the four quadrants. The latter cannot, however, be distinguished from one another at any time during the cleavage, so perfect is the quadriradial symmetry.

Up to the 64-cell stage *Lepidonotus* agrees with *Amphitrite* in the origin and position of every cell: the rosette is always formed in the same manner; the original cross cells proceed to divide in the same peculiar bilateral fashion; the same eight vegetative-pole cells invaginate (some divide again on the surface in *Amphitrite*); the primary prototroch is differentiated at the same time in both forms, and probably from the same cells. *These characters prove beyond question a very high degree of differentiation in the trochophore of Lepidonotus at the 64-cell stage*, even though the quadrants cannot be distinguished from one another. The differentiation from pole to pole is shown by numerous features,— rosette, prototroch, etc.; the bilateral differentiation is proved by the characteristic cross; but the question remains, *which of two planes is the sagittal plane of the embryo?* or, *which of four quadrants is the dorsal one?*

A clue to the solution of the problem is found in forms like *Amphitrite*, where the dorsal quadrant is distinguished not only by the size of its cells, but, after the 64-cell stage, by their characteristic manner of dividing; for example, the mesoderm cell, d^4, always divides bilaterally, and gives rise to the paired mesoderm bands. Unfortunately, it has never been seen to divide in *Lepidonotus* or any other annelid with *equal* cleavage. Again, in *Amphitrite* and *Clymenella*, the interruption in the prototroch is due to the failure of certain cells in the dorsal quadrant to develop cilia, as do the corresponding cells in the

other three quadrants. Now in *Lepidonotus* there is a similar dorsal interruption, and, moreover, the primary prototroch is differentiated at the same time and probably in the same manner as in *Amphitrite* and *Clymenella* (unequal type). If the dorsal interruption in *Lepidonotus* (equal type) arises in the same manner, there must be here also an early differentiation in this quadrant which does not manifest itself in the size of the cells. — But the origin of the *complete* prototroch, like that of the mesoderm, has yet to be ascertained in eggs with equal cleavage. The ascertainment of the origin of one or both of these structures is within reach, and should settle the question whether one of the two blastomeres in equal cleavage is homologous with the larger in unequal cleavage, and the solution of the last question would in turn throw light on the meaning of cleavage and the nature of differentiation, morphological and physiological.

As the matter stands at present, one of two things can be said of the differentiation of the egg of *Lepidonotus* in the 64-cell stage. It has either a complete bilateral organization which is not discernible, or *two* planes of symmetry with four possibilities of orientation, from which it must select one before development proceeds much farther.

b. *Unequal cleavage.*

Eggs with unequal cleavage are characterized by a 4-cell stage with one cell of predominant size. This cell is the same in origin except perhaps in one or two cases.*

The destiny of the other three cells is in most cases very imperfectly known. But the general homology of these early blastomeres is best considered while comparing their more highly differentiated products.

All eggs of this type pass through a characteristic 8-cell

* In Vejdovsky's account of *Rhynchelmis*,[18] the largest cell does not correspond in origin to that in the other forms, but this point needs reinvestigation (cf. Whitman's criticism,[10] p. 126).

In *Physa* (Crampton[20]) the large cell has a different origin, but has the same general fate. It always forms the mesoderm, and at least the larger part of the trunk ectoderm.

stage, consisting of four smaller cells at the animal pole alternating with four larger ones at the vegetative pole. The destiny of these cells in all well-ascertained cases is the same; the four smaller ones together give rise to the umbrellar region of the trochophore, the four lower cells to the subumbrella. When the cells of the upper quartette are comparatively large and divide readily, the umbrella is large and the trochophore active. When, on the other hand, the cells of the upper quartette are smaller and divide less rapidly, the umbrella is smaller and the trochophore less active. *Lepidonotus*, *Amphitrite*, *Nereis limbata*, *Clymenella*, *Nereis Dummerillii*, *Rhynchelmis*, and *Clepsine* form a series of annelids in which there is a gradual decrease in the relative size and karyokinetic activity of the four upper cells, and a corresponding decrease in the size of the umbrella and the activity of the trochophore. The molluscs might be arranged in a similar series, *e.g.*, *Patella*, *Crepidula*, *Unio*, and *Umbrella*.

In the *16-cell stage* the ultimate destiny of four cells, those of the second quartette from the animal pole (trochoblasts), has been completely worked out in *Amphitrite* and *Clymenella*. These give rise to the *primary prototroch* in exactly the same manner in both forms.* Though the worms belong to rather distantly related families (Maldanidæ and Terebellidæ), and the eggs are different in size, in the quantity and quality of yolk, and in the relative size of the trochoblasts, yet the latter agree perfectly in mode of origin and in destiny, and must be placed in the category of homologous cells. The observations in other forms, so far as they have been extended, bear out this homology.—In *Lepidonotus* the divisions of the trochoblasts take place exactly as in *Amphitrite* and *Clymenella*, and when they are completed the trochophore almost immediately begins to swim. The prototrochal cilia are produced, in part at least, by these cells, and a complete correspondence is very probable.

Scolecolepis contrasts sharply with *Lepidonotus*, since it has an almost completely suppressed trochophore. The upper hemisphere, umbrella, is itself very small, while the trochoblasts (?)

* These two annelids are the only ones in which the history of *all the products* of the trochoblasts has been completely worked out.

are conspicuous for their diminutive size and their tardiness in dividing, — just what we should expect of trochoblasts in a suppressed trochophore, if the homology could be extended to this form. Unfortunately, the further history of these cells is unknown.

In *Nereis*, according to Wilson,[1] only twelve of the products of the trochoblasts enter the prototroch, so that the (primary) prototroch consists of four groups of *three* cells each instead of four groups of *four* cells each, as in the case of *Amphitrite* and *Clymenella*. Thus the homology would appear to be somewhat imperfect, but it is sustained by a closer comparison of the behavior of the trochoblasts in *Nereis* with those in *Lepidonotus*, *Amphitrite*, and *Clymenella*. In each, the four trochoblasts arise in the same manner and divide obliquely to the right. In the first three annelids the eight resulting cells again divide obliquely (to the left), while in *Nereis*, according to Wilson, the direction alternates, one cell dividing horizontally, the next vertically, and so on around the egg. It will be readily admitted that *Lepidonotus* and *Amphitrite* have a more typical cleavage than *Nereis*, for they have less yolk and the cleavage is more regular. If we compare the figures of *Nereis* with those of the more regular forms, the so-called horizontal and vertical cleavages show at least a reminiscence of obliquity. In *Nereis* the four products of the trochoblasts which lie nearest the animal pole are said not to enter the prototroch ; in *Amphitrite* and *Clymenella* these cells at first have a similar position, but later certainly form part of the prototroch. In *Nereis*, furthermore, the four cells in question have not been seen to divide again, and we do not know how the prototroch is completed.

I think these facts warrant the assumption that the history of the trochoblasts is the same in *Nereis* as in the other forms.

In *Chætopterus* the trochoblasts divide into sixteen cells in the regular way, but some of them *divide again*, — a fact which is significant, because *Chætopterus* is said to have no prototroch.

Somatoblast. — One other cell in the 16-cell stage, the somatoblast (d^2), is already distinguished from the others by the fact that it contains the anlage of the whole or the greater part of the trunk ectoderm in all annelids where it has been

worked out — *Clepsine*, *Rhynchelmis*, *Nereis Dummerillii*, *Nereis limbata*, and *Amphitrite*, and also in molluscs (cf. Lillie,[3] p. 25). Whether the individual cells of the somatic plate (descendants of the somatoblasts) are homologous in different animals cannot be discussed profitably until we have more data. The only products of the somatoblast whose *exact* origin and destiny are known are, I believe, the teloblasts (neuroblasts and nephroblasts) in *Clepsine* and the paratroch cells in *Amphitrite*. Wilson considers certain of its products in *Nereis* to be without doubt the homologues of the neuro-nephroblasts in *Clepsine*; but it seems to me that the ground for such an homology is extremely insecure (p. 250).

The relationships of the other cells can be more clearly understood by comparing their more highly differentiated products at the 32 or at the 64-cell stage.

32-cell stage, the secondary trochoblasts. — In annelids with a free-swimming trochophore, *Lepidonotus*, *Amphitrite*, *Clymenella*, *Nereis limbata*, etc., the sixteen cells divide at almost the same time into thirty-two cells; they always have the same relative position, forming eight quartettes which alternate from pole to pole. The upper sixteen cells constitute the umbrella, the lower sixteen the subumbrella. a^2, b^2, c^2, and d^2, each divides obliquely into two cells, and it is the destiny of the upper products of the first three ($a^{2.1}$, $b^{2.1}$, and $c^{2.1}$) which now interests us. The cleavage of these secondary trochoblasts has been followed in detail only in *Amphitrite* and *Clymenella*, but in these forms their behavior is similar to an extraordinary degree. The divisions are the same in all three quadrants: each secondary trochoblast divides into four — three large cells and one which is very minute. The former soon acquire cilia and form part of the prototroch, while the minute cell does not become ciliated, and later divides again. A more impressive example of *precise similarity in origin and destiny of cleavage cells* in very late stages could hardly be imagined.

The cleavage of *Scolecolepis*, as far as it has been followed, sustains this homology of the secondary (as of the primary) trochoblasts, for the three secondary trochoblasts ($a^{2.1}$, $b^{2.1}$, and

$c^{2.1}$, Fig. 114) are even more diminutive than the primary, and diminutive trochoblasts are to be expected in suppressed trochophores, on the principle of the homology of cleavage cells.

Wilson ascribes a very different fate to these three cells in *Nereis*. They arise in the same way as in *Amphitrite* and *Clymenella*, and undergo one division in the same manner (the next divisions are not recorded); but two of the cells instead of contributing to the prototroch form the "latero-dorsal region of the trunk" (*l.d.*, text Fig. IV).

I think, however, that the fate of these cells in *Nereis* requires further confirmation, for the following reasons: (1) in *Amphitrite* and *Clymenella* such a fate of $a^{2.1}$ and $c^{2.1}$ is out of the question, because all but a very minute portion of these cells enters the prototroch, and because the latero-dorsal region, in *Amphitrite* at least, is formed from another cell, the somatoblast d^2; (2) in *Nereis* only one division of the cells is recorded, while the origin of the rest of the prototroch remains unsolved; (3) such a fate of these cells involves a serious discrepancy between the behavior of the somatic plate (ventral plate) cells in *Nereis* and in the other forms, while in *Amphitrite* and *Clymenella* — the only forms in which their fate has been accurately ascertained — there is a remarkable agreement.

The stomatoblasts (Nereis) and larval mesoblast (Unio). — In *Nereis* Wilson records the peculiar fate of the sister cells of the secondary trochoblasts, *i.e.*, of the cells $a^{2.2}$, $b^{2.2}$, $c^{2.2}$. They form a ring about the stomodæum and hence are called *stomatoblasts*. But Lillie shows that in *Unio* $a^{2.2}$ has an equally peculiar fate: it forms the *larval mesoblast*. The inference has been drawn and was explicitly stated by Lillie that in this case cells of the same origin have different destinies, — a blow to cell homology (Lillie,[3] p. 37). Stated in this way, that $a^{2.2}$ is a stomatoblast in *Nereis* and the larval mesoblast in *Unio*, the inference is well warranted. But, if we examine more critically the fate of these cells in both forms, an obvious fallacy appears. Wilson does not describe in detail the cleavage of the cell in question, though he shows that it divides at least once in the same direction as in *Amphitrite* and *Clymenella*. Only one product of

this division becomes the *stomatoblast*. What becomes of the other product? In *Unio* (p. 24, Figs. 42–47) Lillie describes two or three small cells as "budded off" from $a^{2.2}$ before the latter sinks into the interior to form the larval mesoblast. In other words, $a^{2.2}$ *divides into a group of cells, one of which is the actual larval mesoblast*. What becomes of the others?

There is no reason for believing that the product of the division of $a^{2.2}$, which in *Nereis* becomes a stomatoblast, corresponds in origin to that which in *Unio* becomes the larval mesoblast. But there is excellent reason for believing that it does not, for it is the *lower* product of the first division which in *Nereis* forms the stomatoblast, and the *upper* product which in *Unio* gives rise to the larval mesoblast.

The transition to the 64-cell stage. — The rest of the cells of the 32-cell stage have the same origin and position in the various forms under consideration, but the examination of their morphological relations will be facilitated by comparing their products after the next cleavage, namely, at the 64-cell stage. The transition from thirty-two to sixty-four cells is itself of interest when one compares *Nereis* with more regular forms.

In *Lepidonotus*, *Amphitrite*, *Clymenella*, and *Chætopterus* all the thirty-two cells divide in a left-oblique direction, according to the regular rhythm, and, except in *Chætopterus*, so nearly at the same time that an actual 64-cell stage results.

Wilson divides the cleavage of *Nereis* into a 38, a 42, and a 58-cell stage, but the fact that in this annelid the division of the cells is not synchronous need not confuse us. In *Lepidonotus*, *Clymenella*, *Amphitrite*, and *Chætopterus* all thirty-two cells divide in the left-oblique direction, and it would seem from Wilson's figures, that the same thing may be said of *Nereis* (of course excepting A_3, B_3, and C_3, which do not divide at all). Therefore Wilson's statement that the *transition from the "spiral"* (oblique) *to the bilateral type of cleavage occurs at this stage*, and the consequent inferences, may be questioned.

The 64-cell stage. — We will begin our comparison with the cells at the animal pole. The *apical rosette*, consisting of four very small cells, arises in exactly the same way in *Nereis*, *Spio*,

Aricia, *Polymnia*, *Amphitrite*, *Clymenella*, *Chætopterus*, and *Lepidonotus*. Conklin finds a similar rosette in the mollusc *Crepidula*, and Heymons mentions it in *Umbrella*, but gives no figure. Lillie did not follow the cleavage of the apical cells in *Unio* far enough to find out whether the rosette existed or not. In *Amphitrite* and *Clymenella* all four rosette cells divide again in the regular reverse oblique direction, and Wilson figures one of these cells as dividing in this way in *Nereis*. Their division in other forms has not been recorded. In *Lepidonotus* it is certain, and in *Amphitrite* and *Nereis* extremely probable, that the rosette cells, or their products, bear the cilia of the apical tuft. The rosette cells, therefore, seem to have the same origin and fate.

The four cells which alternate with the four of the rosette are the parent cells of the *cross*, a peculiar pattern first described by Wilson[1] in *Nereis*, *Polymnia*, and *Aricia*. I have found an exactly similar cross in *Amphitrite*, *Clymenella*, and *Lepidonotus*. The cleavage of the four parent cells departs so abruptly from the method of the earlier cleavage, and is so similar in all annelids where the cross occurs, that it deserves special mention. The cross has been described in representatives of seven genera belonging to six different families; in *Spio*, *Polymnia*, and *Aricia* without figures, so that we will confine our comparisons to *Nereis*, *Lepidonotus*, *Amphitrite*, and *Clymenella*. In all of these the four parent cells arise in the same way and lie symmetrically, two on either side of the middle line, one in each quadrant. Each divides horizontally and bilaterally, so that the pattern on one side is the counterpart of that on the other, and I have searched in vain for any reminiscence of oblique cleavage. The outermost cells in each arm soon divide again in the same direction, making three cells in a row.

Peculiar interest attaches to the middle cells of the dorsal arms, because their destiny has been ascertained in *Nereis* and *Amphitrite*. In *Nereis* they do not divide again, but sink into the interior of the egg and become what Wilson provisionally calls "head-kidneys." In *Amphitrite* also they sink beneath the surface, except for a slender stalk, and form a pair of immense unicellular mucous glands. Although

the function of these cells in *Nereis* is not definitely known, they are doubtless homologous with those in *Amphitrite*, since they are the outcome of such peculiar and exactly similar cleavages, and do not divide again, but become specialized unicellular organs. In other forms we know nothing of the ultimate destiny of these cells; they have never been observed to divide and they vary in size, being quite small in *Clymenella*.

The middle cells of the ventral (anterior) arms divide meridionally both in *Nereis* and in *Amphitrite*. In *Nereis* we know nothing further of their destiny, but in *Amphitrite* they show a decided tendency to sink below the surface like the cells of the dorsal arms; and, although I have not followed their history, it is certain that they correspond exactly in *position* to two pairs of ventral unicellular mucous glands.*

Several further divisions of the cross have been ascertained in *Nereis*, *Amphitrite*, *Clymenella*, and *Lepidonotus*. As far as known, the divisions are always strictly bilateral, in the same direction, and preserve their tendency towards quadriradial symmetry in all four quadrants; but the pattern of the cross becomes indistinguishable after a few divisions.

Wilson says that in *Nereis* "there can be no doubt that the cross gives rise in large part to the cerebral ganglion." It would appear from its position in *Amphitrite* that it does contribute to the formation of the ganglion, but I think that in both cases the fate of the cross cells, excepting, of course, the head-kidney and mucous glands above referred to, is doubtful.

We do not know the particular fate of the cells which lie between the arms of the cross, though in *Amphitrite* some of those lying in the dorsal quadrant pass through the interruption

* If the inference that these cells become the mucous glands is correct, indeed, if it is not, we have here a good illustration of what may be called a quadriradial symmetry in the trochophore, especially in the umbrella; a symmetry by virtue of which phenomena occurring in one place, not only repeat themselves on the opposite side of the sagittal plane, but in each of the other three quadrants. This tendency is traceable in the cleavage in the umbrellar region and in the distribution of the purely larval organs, prototroch, mucous glands, etc. In a form like *Lepidonotus*, the quadriradial symmetry is apparently perfect, but in forms with unequal cleavage it appears to be gradually giving way to the strictly bilateral type as illustrated by the series *Amphitrite*, *Nereis limbata*, *Clymenella*, and *Nereis Dummerillii*.

of the prototroch, and contribute to the ectoderm of the subumbrella (p. 239).

The cells of the prototroch have already been considered, and this brings us to the lower hemisphere, or subumbrella. While the somatic plate as a whole has a similar origin and fate in the various annelids, we have not data enough to determine whether the homology may be extended to the individual cells. It seems probable that the "neuroblasts" and "nephroblasts" of *Clepsine*, *Rhynchelmis*, and *Lumbricus* are homologous.

Wilson believes, moreover, that the *proteloblasts* in *Nereis*, are the homologues of the *neuro-nephroblasts* in *Clepsine*, but I find nothing in *Amphitrite* to support this view.

The early divisions of the cells of the somatic plate have a striking similarity in the various forms. One cell in particular is conspicuous for its similarity in *Nereis*, *Amphitrite*, *Clymenella*, and *Unio*. It lies in the lower right-hand corner of the somatic plate (marked $x^{1.2}$): it gives rise to part of the proctodæum in *Amphitrite*. A comparison of the exact origin of the paratroch in the various forms would be of greatest interest.

The secondary trochoblasts have been discussed. As to the rest of the ectoderm on the lower hemisphere, we know only the general fate of groups of cells. In *Nereis* Wilson says that the *terminal cells* which form the proctodæum are "certainly in part the offspring of the primary mesoblast," but this cannot be said of *Amphitrite*.

We have records of the origin of the mesoderm in the following forms: among annelids, *Clepsine*, *Nereis Dummerillii* (free-swimming and suppressed trochophore), *Nereis limbata*, *Spio*, *Aricia*, *Polymnia*, *Amphitrite*; among molluscs, *Neritina*, *Planorbis*, *Umbrella*, *Unio*, and *Crepidula;* among polyclades, *Discocœlis*. A comparison of the mesoblast formation in these forms yields additional evidence for the homology of cleavage cells. In all except *Clepsine*, *Nereis Dummerillii*, and *Discocœlis*, the original mesoderm cell arises from the same one of the fourth generation of cells, which has descended from *D* of the 4-cell stage. *The unpaired mesoderm cell belongs to the ideal 64-cell stage, and is one of the second quartette of cells counting from the vegetative pole, and always arises by a left-oblique*

cleavage. The next furrow always divides this cell equally into a right and a left mesoderm cell.

Clepsine, as described by Whitman,[10] fails to accord with the above in that the original mesoderm cell is differentiated at the ideal 16-cell stage instead of at the 64-cell stage, but Dr. Whitman tells me that certain small cells are budded off before the bilateral division of the mesoblast. It is possible, therefore, that the origin of the mesoblast cell in *Clepsine* is not exceptional.

I fully concur with Wilson in his criticism of the origin of the mesoblast in *Nereis Dummerillii*, as recorded by Goette and von Wistinghausen. Goette's[12] account of the cleavage is incomplete, and von Wistinghausen[5] probably overlooked a cell, and thus threw the responsibility of mesoblast formation upon the wrong one (cf. Wilson,[1] p. 435).

According to Lang[15] the origin of the mesoderm in *Discocælus* differs from that in the annelids and molluscs in at least two important respects: it arises not from one, but from all four quadrants, and from cells of two generations, both earlier than that giving rise to mesoderm in the annelids, etc.

In annelids these two generations of cells form a part of the ectoderm of the subumbrella, including a portion of the prototroch. Granting, for a moment, the correctness of Lang's observations, we find ourselves in a dilemma well stated by Wilson,[1] p. 448: "Unless, therefore, we are prepared to maintain the absurd proposition that the mesoblast of the polyclade is homologous, not with the mesoblast of the annelid, but with the ectoblast of the lower hemisphere (including, of course, the ventral plate with the ventral nerve cord), we cannot escape the conclusion that exact equivalence of embryological origin is not a proof of homology, so far at least as the cleavage stages are concerned." Again, in his lecture on "The Embryological Criterion of Homology,"[22] Wilson says: "We find (in the polyclade) a cleavage very closely resembling the annelid type in form, yet the individual blastomeres have from the very start an entirely different morphological value." Lillie[3] (p. 37) mentions the mesoblast of *Discocœlis* as affording another instance of cells of different origin which have the same destiny.

However, I am not convinced that the cells described by Lang do give rise to the mesoderm, and I believe it possible that the mesoderm is formed in the same manner and from exactly the same cell as in the annelids with unequal cleavage.

Lang figures the cleavage up to about the 64-cell stage. At this time the cells which, according to his description, become the mesoderm, lie upon the surface, and even extend slightly over the ectoderm. To determine the exact cell lineage of any organ requires a close and uninterrupted series of observations. The stages from 64 cells to the end of gastrulation, are, of course, the critical stages for establishing the origin of the mesoderm. Lang does not record, either in text or in figures, any observations covering this important period.

We now naturally turn our attention to those cells in *Discocœlis*, which in other animals, with this type of cleavage, regularly give rise to the mesoderm bands. Lang's Fig. 12, Plate XXXV, of the lower pole of *Discocœlis* corresponds in every essential point to the ideal 32-cell stage of annelids and molluscs with unequal cleavage. All the cells have the same origin and relative position, and belong to the same generation. The four vegetative cells are comparatively large, and one exceeds the others in size. Now in the annelids these four cells regularly divide upon the surface; the eight resulting cells are of the same generation and lie in a characteristic position, four meeting at the vegetative pole and four alternating with them. One of the latter, the offspring of the *larger cell* (*d* quadrant), is always the mesoderm cell, and lies in the *middle line of the future body*, while the other seven are entodermal. In *Nereis* only the larger cell divides on the surface, so that the entoderm has but four instead of seven cells; a fact which only emphasizes the significance of the division giving rise to the mesoderm cell. In all forms where its origin is known, the mesoderm cell begins at once to behave in a perfectly unique and characteristic way. — It always divides into two equal cells, one of which lies on the right and one on the left of the middle line. These cells give rise to the right and left mesoderm bands respectively.

When Lang's figures and text are examined in the light of this comparison, the correspondence of *Discocœlis* to the other forms is complete in every detail: so complete as to be fairly startling. The eight cells are formed in the same manner (Figs. 12–16). The one which corresponds to the mesoderm cell in the other animals, divides bilaterally, and one product lies on either side of the middle line (Fig. 17, a_1, a_2, Pl. XXXV, text Fig. XXV, p. 340).

Since in a large series of forms the mesoderm cell is the same in origin (same generation and position), and immediately divides in a manner different from all the other cells, and since a cell in *Discocœlis* corresponds exactly in origin and begins the same characteristic career, I believe it may be the mesoderm cell in *Discocœlis* also.

In many cases the paired mesoderm cells divide while still at the surface. The ventral or "anterior" products, whose exact fate is uncertain, may be as large as the posterior (*Clymenella*), or much smaller, as is usually the case (*Nereis, Unio, Umbrella, Spio, Aricia, Crepidula*). In *Spio* and *Aricia* Wilson says the anterior products "are very minute and appear to be rudimentary." In *Amphitrite* they are extremely minute, arising at a later period by a cleavage which seems to defy mechanical conditions, and are carried into the segmentation cavity and lie at the anterior ends of the mesoderm bands. In *Polymnia* this "preliminary superficial budding seems not to take place."

We cannot interpret this peculiar division until more is learned about the exact fate of these cells. The fact that they are sometimes of large size and divide readily, while at other times they are much smaller or exceedingly minute and are slow in dividing, would seem to indicate that they are the anlage of some variable or rudimentary structure.

Entoderm. — The cytogenetic origin of the entoderm is another example of cell homology. In many forms the larger of the four cells at the vegetative pole in the 32-cell stage divides, the outermost product giving rise to the *mesoderm cell*, while the rest of the products are *entodermal*, whether they divide again on the surface or not. In *Nereis* the entoderm

plate is composed of four cells: in other forms three of these divide on the surface and give rise to an entoderm plate of seven cells. This appears to be the case in the molluscs, *Planorbis*, *Neritina*, and *Umbrella*. In *Lepidonotus* the same number of divisions obtains, but we do not know how many or what cells are mesodermal. In several annelids, *Polymnia*, *Aricia*, *Amphitrite*, *Clymenella*, the four inner cells of the plate divide again, making the entoderm plate number eleven cells.

The ova of polychætous annelids, and of many other forms in related phyla pass through cleavage stages easily referable to a common type; the regularity and topographical peculiarities of these stages have made it possible to compare the corresponding cells among the various animals in all stages of cleavage, while the very early differentiations of the blastomeres often makes this comparison of paramount theoretical value. The more extensive and thorough the comparison, the better warranted the inference that there exists between the cleavage cells of various forms the same morphological relationship which exists between adult organs and tissues, *i.e.*, *homology*.

The main objections urged against cell homology in this type of cleavage are the alleged difference in the fate of the cell $a^{2.2}$ in *Nereis* and in *Unio*, and the difference in origin of the mesoblast in *Discocœlis* and in the other forms. On closer scrutiny, these objections lose their force because of a seeming oversight in the first instance, and the lack of evidence in the second.

In attempting to explain annelid cleavage by means of the mechanical principles already deduced from experimental sources and applied with varying success to other forms of cleavage, two points present themselves: first, in the echinoderms, vertebrates, etc., very little has been ascertained concerning the normal relations between the cleavage cells and the organs and tissues of the adult of the same individual, and between the corresponding cleavage cells of different species; second, in the annelids, etc., while considerable is known concerning both of these normal relations, no successful experiments have been

made which enable us to analyze the causes of these relations into mechanical components. What is to be said at present about these components must be deduced from a comparison of normal cleavage in different forms; often where cleavage *processes are similar*, the mechanical *conditions are different;* and, where the *conditions are similar*, the *processes are different.*

II. Cleavage Considered from the Point of View of Developmental Mechanics.

The doctrine of the mechanical causes of organic forms must, when applied to annelid cleavage, account for (1) the relative size of the cells, (2) the direction of the cleavage, and (3) the time or rate of cleavage, for all three are potent factors in determining the specific form of the trochophore.

Relative size of the cells.— Aside from the very unequal cleavage of the *oöcytes* in the formation of the polar globules, the first cleavage of the egg itself is sometimes equal, sometimes unequal. The explanation has been offered that the inequality is due to the influence of yolk; that the karyokinetic apparatus is not able to effect a cleavage through the middle in cases where the yolk is abundant. A comparison of *Lepidonotus*, which has little yolk and equal cleavage, with *Amphitrite* and *Clymenella*, which have more yolk and unequal cleavage, seems to sustain this explanation; but a further comparison with other forms shows that the influence of yolk cannot be considered the chief determining factor of the relative size of cells. The egg of *Chætopterus*, for instance, is about the size of that of *Lepidonotus* and has apparently the same amount of yolk, yet it divides very unequally. The eggs of *Crepidula* have a very large amount of yolk and are much larger than *Unio*, yet the first cleavage of the former is nearly equal, that of the latter very unequal. In the later cleavage stages, cells with a large proportion of yolk are as likely to divide equally as unequally. Examples are numerous. In *Amphitrite* the smaller of the first two blastomeres, though it arises by an unequal division, divides equally, while each product of this cleavage divides *unequally*. On the other hand, the largest

cell of the 8-cell stage arises by an unequal division, but divides about *equally* (D_2 and d^2, Pl. X). The divisions corresponding to the latter in the other three quadrants are extremely unequal, though the relative amount of yolk in the cells is less. The first division of the original mesoderm cell in *Amphitrite* is equal, the division of each product extremely unequal. In *Clymenella* the original mesoderm cell divides equally, and the daughter cells also.

Cells without much yolk divide equally or unequally without regard to size, as may be seen by simply examining the figures of the upper hemisphere in the various eggs at all stages, especially those representing the formation of the rosette, cross, and secondary prototrochal cells in *Amphitrite* and *Clymenella*.

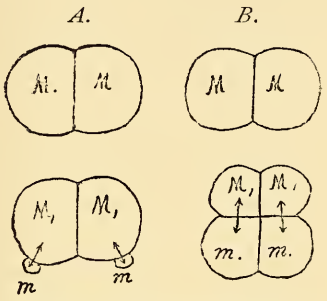

FIG. XXII.—Shows the relative size of the products of the paired mesoblasts, *M* and *M*, after their first division; *A*, *Amphitrite*; *B*, *Clymenella*.

As to the influence of gravity in determining the size of the cells, it needs only to be said that many eggs, *i.e.*, *Amphitrite*, *Clymenella*, and *Nereis*, develop normally whether in one position or another.*

Similarity in *position* of cells does not insure similarity in the relative size of products. In the 32-cell stage of *Lepidonotus*, four cells lie at the animal pole in the same position, with reference to surrounding cells, as the four cells at the vegetative pole. The eight are of about the same size, and divide unequally in the same direction and at the same time; but at the animal pole the resulting central cells are the *smaller* products, while at the vegetative pole the central cells are the *larger* products. In *Discocœlis* the four vegetative cells have the same position as in *Lepidonotus*, but the four central products of the next division are the *smaller*. The division of the paired mesoderm cells and the secondary trochoblasts in *Amphitrite* and *Clymenella* furnish additional illustrations (text Figs. XXII, XXIII).

* Dr. W. M. Wheeler tells me that the same is true of the egg of *Blatta*.

The direction of cleavage. — Mechanical principles which are apparently sufficient to explain the direction of cleavage in one instance are totally inadequate in others. Thus, when we have decided that certain factors determine the horizontal direction of the first cleavage spindle, for example, the principle of equal or least resistance due to the segregation of yolk, these factors must be considerably modified when called upon to explain the *vertical* position of the two maturation spindles; for the conditions, segregation of yolk, etc., are the same.*

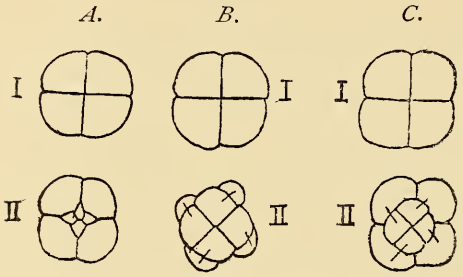

FIG. XXIII. — *A*, *Lepidonotus*: *I*, apical cells; *II*, their products. *B*, *Lepidonotus*: *I*, vegetative-pole cells; *II*, their products. *C*, *Discocœlus*: *I*, vegetative-pole cells; *II*, their products.

The appearance of the peculiar lobe in *Chætopterus*, simultaneously with the first cleavage, seems to indicate that the cleavage does not take place in the direction of *least resistance*, else it would coincide with the axis of the lobe, as in Loeb's [26] experiments on sea-urchin eggs.

In this type of cleavage the spindles of the two blastomeres are inclined to each other, and almost always in the same direction. Why is this? And why does *Physa* form an exception to the rule?

In *Lepidonotus*, *Amphitrite*, and *Clymenella* from the division of the first two blastomeres up to the "ideal" 64-cell stage the regular alternation in the direction of cleavage accords with the "law" that successive cleavage planes tend to lie at right angles.† After the ideal 64-cell stage many of the cells divide

[26] J. LOEB: On Some Facts and Principles of Physiological Morphology. *Biol. Lectures*. Woods Holl, 1893.

* Watasé,[23] in his "Studies on Cephalopods," *Journ. of Morph.*, vol. IV, no. 3, figures an egg of *Loligo* in which the blastoderm is developing on the side instead of on the end of the egg. The pattern of the cleavage is the same as in the normal egg, though the conditions must be decidedly different.

† In the retarded cleavage on the upper hemisphere of *Umbrella* there are certain divisions which do not agree in direction with the corresponding

without the slightest regard to this rule of alternating cleavage. Their only concern seems to be to form a pattern bilaterally symmetrical with respect to the sagittal plane of the embryo. This tendency is well illustrated in the formation of the *cross* in all forms where the latter occurs; in the characteristic first division of the mesoderm cell (d^4), even in *Discocœlis;* and in the further division of the somatic-plate cells (p. 241). These cleavage phenomena present three general features which are to me especially interesting: (1) certain cells divide bilaterally, while their adjacent sister cells continue to divide in the alternating oblique rhythm; (2) the former cells *suddenly* disengaged themselves from the influence, whatever it may be, which compels the parent cells and sister cells to divide in the alternating rhythm; (3) in most cases the cells which so promptly respond to the influence of bilaterality, so to speak, are the anlagen of bilaterally symmetrical structures, *e.g.*, mucous glands (*Amphitrite*), head-kidneys (*Nereis*), mesoderm, somatic plate, etc.

The direction of the first division of the paired mesoblast cells in *Amphitrite* stands in direct contradiction to the "law" that the spindle lies in the direction of least pressure or that it lies in the long axis of the protoplasm of the cell, for it coincides with the direction of greatest pressure, and is at right angles to the long axis (cf. p. 247). Additional importance attaches to this division, for, from the point of view of cell homology, it is a reminiscence of a surface division which still persists in some forms; and thus we have here a contest between the influence of mechanical surroundings and an intrinsic tendency to divide in a certain direction, at a certain time, and the latter prevails.

The "parent cross-cells" in *Chætopterus*, on the one hand, and in *Nereis*, *Lepidonotus*, *Amphitrite*, etc., on the other, afford remarkable examples of cells of similar origin and position dividing in different animals in fundamentally different direc-

division in the above forms. Why these divisions are retarded, and what is the destiny of the cells, are, unfortunately, unsolved problems. It looks, however, as though the direction of the cleavage was influenced by the position of the surrounding cells, and, therefore, may accord fairly well with the mechanical hypothesis.

tions. The alternating oblique direction of the division of these cells in *Chætopterus* compared with the strictly bilateral division in all the other forms makes it doubly difficult to explain either case on mechanical principles.

I have already given my reasons for believing that Wilson is mistaken in asserting that the *bilateral period* in *Nereis* begins with the 38-cell stage. It certainly begins only after the 64-cell stage in the forms I have studied. I disagree further with Wilson[1] when he says that the *cause* of the introduction of bilateral cleavage is the reduction of the "posterior macromere" to the size of its fellow. Bilateral cleavage occurs in *Lepidonotus* and in *Umbrella* where there can be no such "reduction," because the macromeres are equal from the first. Moreover, in *Chætopterus*, where the posterior macromere *is* larger, certain bilateral divisions which are always found in the other forms do not occur.

I do not understand how Kofoid could have inferred from Wilson's or Heymons's figures or text that the rule of alternating (spiral) oblique cleavage holds good throughout the cleavage of *Nereis* up to the "58-cell stage," and of *Umbrella* up to the 91-cell stage. The 58-cell stage in *Nereis* is brought about by the obviously bilateral division of some of the *cross cells* and of the mesoderm cells, while the 91-cell stage in *Umbrella* is attained only by the bilateral division of the mesoblast.

The rate of cleavage. — The absolute rate of cleavage varies with the temperature of the water and among different species, and cannot be predicted from the size or physical appearance of the egg (cf. *Amphitrite* and *Unio*). In all polychætes which I have studied, the division of the oöcytes of the first order, *i.e.*, the formation of the first polar globule, will not take place until the sperm has entered the egg. Just how the entrance of the sperm stimulates the cell to karyokinetic activity is unexplained, but the phenomenon is suggestive in that it shows that, in one case at least, it is not the mass of the egg nor the presence of more or of less yolk that determines the *time* of cell division, but a stimulus of some kind, analogous, perhaps, to that which starts into activity the motor apparatus of pigment cells, leucocytes, or muscle cells.

When the cleavage is once started, it is evident that the form of the embryo is conditioned by the relative rapidity of cell divisions in certain regions, as well as by the relative size of the cells and the direction of cleavage.

A close comparison of the rate of cleavage of corresponding cells in the eggs of various annelids, molluscs, etc., and of the rate in different cells in adjacent areas of the same egg, clearly demonstrates that neither the effect of gravity, the size of the cells, their position, nor all these together are sufficient to account for the rate of cleavage. As to the effect of gravity, it is only necessary to repeat that many eggs develop normally in any position. Let us test the other factors in a number of instances. In *Nereis* the smaller of the two unequal blastomeres regularly divides first ; in *Chætopterus*, on the other hand, though unequal, both blastomeres divide at exactly the same time. I have examined this egg with particular care by means of sections, and find that at successive stages of karyokinesis even the daughter centrosomes at the ends of the spindles are just as far apart in one cell as in the other. In *Amphitrite*, also, the two cells divide at about the same time, — sometimes, however, the larger one is slightly in advance.

In many forms the division of the first eight cells takes place synchronously, whether the cells are of nearly the same size and contain little yolk, as in *Lepidonotus*, *Eupomatus*, etc., or whether, as in many other forms, of which *Scolecolepis* is perhaps the best example, the four upper cells are comparatively small and free from yolk, while the four lower cells are large and full of yolk.

The mesoderm cell, in several forms, manifests great karyokinetic activity, while the entoderm cells, which correspond exactly in origin, but lie in the other quadrants, cease dividing temporarily, — yet in size and amount of yolk the mesoderm cell often very closely resembles those of the entoderm. The cells of the upper hemisphere in *Unio* are tardy in division, but are of moderate size and, to all appearances, similar to the corresponding cells of *Amphitrite*, *Nereis*, etc.

The consideration of the rate of cleavage must include not only cells which are precocious or tardy, or cease to divide tem-

porarily, but also those which suddenly stop dividing altogether. For example, the cells of the primary prototroch in *Amphitrite*, *Clymenella* and *Nereis;* the secondary prototroch cells in *Amphitrite* and *Clymenella;* the "head-kidneys" of *Nereis;* the mucous glands and paratroch cells in *Amphitrite*. These cells possess no peculiarity of size, yolk segregation, nor position, which can account for their sudden disinclination to divide. The neighboring cells, which continue to divide, all share the same characteristics, as far as size and yolk are concerned; and, as for position, in *Chætopterus* the "prototrochal cells" *do* divide again, — at least some of them.

The conceptions of the mechanical interaction of cleavage cells as units of mass are too crude to offer any explanation of these complex phenomena.

The cytogenetic origin of the prototroch and paratroch forms an interesting study in cell differentiation, since these organs are of great morphological significance and high physiological specialization, and since their exact origin cell by cell from the ovum has been accurately ascertained. Thus, for example, if we apply a "theory of determinants" to these cases, it would appear that the determinants of the *primary prototroch* are all segregated in the four "trochoblasts" of the 16-cell stage, for, though each of these four cells divides *twice more*, *all* the resulting cells are *prototrochal*.

The *secondary trochoblasts* (*Amphitrite* and *Clymenella*), on the contrary, contain other than prototrochal determinants. Each divides into two cells, of which one contains only *purely prototrochal* elements, while the other contains determinants of other structures also, and its division results in three prototrochal cells and one cell which becomes part of the general ectoderm.

When we trace back the history of the determinants of the primary and secondary trochoblasts respectively, we find that they are already separated in the 8-cell stage. At this stage the former are contained in the four upper, the latter in three of the lower cells. When these determinants control certain cells, even though the latter belong to different generations, they all unite to form one organ, the prototroch. However,

there is a good deal of symmetry in the formation of this organ, since each quadrant contributes exactly the same number of cells and in the same manner, except that the dorsal quadrant furnishes no secondary prototrochal cells.

On the other hand, in *Amphitrite* the determinants of the paratroch — a strictly bilateral organ — are all within one cell, the somatoblast, of the 16-cell stage, and at the next division the determinants of *three* of the four cells of the paratroch come to lie in one of the resulting cells, and those of the fourth in the other. Though the succeeding cleavages of these two somatoblast cells are quite different, nevertheless, after several divisions, the determinants come together again in control of four cells, which are bilaterally placed with respect to the larva and belong to the same generation, — the eleventh, counting the ovum as the first. That this is significant is indicated by the fact that two extremely minute cells are "budded off" just before the complete differentiation of the paratroch. Otherwise, of course, two of the cells would belong to the tenth generation.

When the origin of the prototroch and of the paratroch has been ascertained in a larger number of forms, we shall be in a position to discuss whether the destiny of cells is conditioned upon their contained determinants or upon their position.

III. Axial Relations.

The discussion of the axial relations of the egg of *Amphitrite* naturally falls into two chapters: the *orientation of the egg* with respect to the future sagittal plane, anterior and posterior end of the larva, etc.; and the *regional metamorphosis* or shifting of areas which occurs in consequence of the transformation of the one-layered blastula into the three-layered larva.

Nothing is known of the orientation of the egg until the first maturation spindle is formed; the direction of this indicates the future position of the polar globules and these in turn indicate the position of the anterior end of the trochophore, or the animal pole of the egg; 180° opposite lies the vegetative

pole. A line connecting the two poles is the egg axis. We can distinguish neither the prospective sagittal plane, nor the right and left sides of the trochophore, until the first cleavage spindle appears. This spindle indicates the direction and position of the first furrow, and all the other furrows follow in perfectly regular and constant sequence. At the 4-cell stage the prospective sagittal plane cuts *B* and *D* as in most annelids,— *Clepsine*, *Rhynchelmis*, *Clymenella*, *Aricia*, *Polymnia*, etc.; and in *Unio* and *Discocœlis*. The cells *A*, *B*, *C*, and *D* are the anlagen respectively of the left, ventral, right, and dorsal quadrants of the trochophore. Therefore, the second cleavage furrow does not coincide with the sagittal plane of the embryo.

When the four cells divide into eight, the upper quartette alternates with the lower so that the second cleavage furrow is twisted and its course more nearly coincides with the sagittal plane on the upper than on the lower hemisphere. From this time the number of cells increases in geometrical progression up to sixty-four, without effecting any considerable redistribution of material. The sixty-four cells are disposed in sixteen alternating quartettes, and the egg is capable of very precise orientation with respect to the sagittal plane, anterior and posterior ends, ventral and dorsal, right and left sides.

Now, if the second furrow is followed around the whole egg, its course is found to be an irregular zigzag, but its general direction is at a considerable angle to the sagittal plane. The embryonic material is segregated in such a way that the mesoderm is contained in one cell, the entoderm in seven; of the fifty-six remaining ectoderm cells, sixteen are already functioning as prototroch and four constitute the somatic plate. The prototrochal cells naturally divide the embryo into an umbrellar and a subumbrellar hemisphere. On the latter the principal shifting of areas, which characterizes the regional metamorphosis, takes place. The regions which figure as units during the transformation are the somatic plate, the mesoderm and entoderm, and the remaining subumbrellar ectoderm.

The main features of the metamorphosis are as follows: the mesoderm and entoderm sink into the segmentation cavity, and the somatic plate thins out and extends over the large area thus

vacated, besides still occupying its original territory. The manner in which this extension takes place is significant. — Except for a slight early movement of the plate backward, so that its edge reaches the vegetative pole in the middle line, all this extension is lateral and the material moves only in the arcs of small circles parallel to the prototroch or equator of the egg (p. 250). Thus, of course, the material just at the vegetative pole does not move. The lateral edges of the plate on either side come together and concresce in the mid-ventral line, and separate the blastopore or stomodæum from the posterior end.

As a result of the metamorphosis, the entoderm and mesoderm pursue their respective destinies inside the segmentation cavity, while the somatic plate occupies the greater part of the area of the subumbrella, including, of course, the posterior end, the entire dorsal and lateral region (excepting a small tract just behind the interruption of the prototroch, in the mid-dorsal line, occupied by cells from the umbrella), and a large portion of the ventral area: all the material lies practically in the same *latitude* as at first. At this point a comparison of *Amphitrite* with *Nereis* is of special interest: —

With regard to the axial relations, Wilson maintains two theses upon which he bases many far-reaching interpretations.

First, the second cleavage furrow coincides with the future sagittal plane of the embryo. Since one of the resulting cells is larger than the others, the embryo is one-sided in the early cleavage, and the cause of the transition from the spiral (oblique) to the bilateral cleavage is the reduction of this posterior macromere to the size of its fellow on the opposite side (Wilson,[1] p. 445). Now in *Amphitrite* and *Clymenella* the second furrow is inclined to the future sagittal plane so that the organism may be considered bilateral from the start, and the introduction of bilateral cleavage must be referred to an entirely different cause (cf. p. 241). And at no time does the second cleavage furrow, as a whole, even approximately separate the material of the right and left sides of the embryo. Since the cleavage of *Nereis* is essentially like that of these forms, the coincidence of the second cleavage furrow with the sagittal plane may well be questioned. The error seems to be in mis-

taking a small part of the furrow, that between the entomeres, for the whole (cf. Lillie, p. 42).

Second, during the regional metamorphosis, the "ventral plate" shifts through an angle of about 90°, so that the main portion occupies the ventral region of the trunk, and the teloblastic area, which lay in the dorsal region near the prototroch, comes to lie at the posterior pole. This leaves the latero-dorsal and mid-dorsal areas to be accounted for in another way.

From this thesis Wilson deduces his theory of the shifting of the neural axis, and of the homology of the posterior teloblasts with the neuro-nephroblasts of the Oligochætes and Hirudinea, and elaborates a scheme for harmonizing the apparently irreconcilable metamorphosis of various larval forms,— *Polygordius*, *Clepsine*, *Lumbricus*, *Lopadorhynchus*, etc. (Wilson,[1] p. 426). The phenomena of axial metamorphosis in *Amphitrite* are utterly at variance with the terms of this second thesis, and therefore do not substantiate the interpretations which rest upon them (cf. p. 250, text Fig. IV).

To return to *Amphitrite*. During the regional changes on the lower hemisphere, the four paratrochal cells, which at first lay in a row at the posterior lip of the blastopore, are brought round to form a circle near the posterior end of the larva, parallel with the prototroch; and the cells of the somatic plate, concrescing in the mid-ventral line, separate the paratroch from the stomodæum. From this time the trochophore elongates in the direction of the original egg axis, by the division of the cells of the budding zone, which lie anterior to the prototroch; the latter persists until the trunk develops several metameres, and always belongs to the ultimate segment of the body.

Few changes take place upon the anterior hemisphere which affect the axial relationships of the larva. The dorsal umbrella mucous glands, whose cytogenetic origin is known, may be relied upon as orienting points until the larva has several setigerous metameres; and the median tentacle, since it bears a constant relation to the mucous glands, orients this region after their disappearance.

University of Chicago,
May, 1895.

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REFERENCE LETTERS.

The quadrants of the egg are indicated by the letters *a*, *b*, *c*, and *d*. The cells at the two poles of the eggs are distinguished by these letters with figures as subscripts, *e.g.*, A_3, B_4, etc., at the vegetative pole, $a_{1.2}$, $b_{1.2}$, etc., at the animal pole: for the other cells the figures are written as exponents, *e.g.*, $a^{2.1}$, $b^{3.2}$. In general, the upper product of a cell division receives the smaller exponent, thus:

$$a^2 \begin{cases} a^{2.1} \begin{cases} a^{2.1.1} \\ a^{2.1.2} \end{cases} \\ a^{2.2} \begin{cases} a^{2.2.1} \\ a^{2.2.2}\ * \end{cases} \end{cases}$$

The following abbreviations are also used: †

*,	landmark, first seen in Fig. 35.
bp.,	blastopore.
ent.,	entoderm.
gl.l.,	left dorsal umbrellar mucous gland.
gl.rt.,	right " " " "
gl.,	other umbrellar mucous glands.
l^1, l^2, l^3,	cf. reference to Fig. 34.
mem.,	egg membrane.
M,	mesoderm proteloblast.
m m,	anterior products of first division of paired mesoblasts.
M M,	paired mesoderm cells, or teloblasts of mesoderm bands.
mes.,	mesoderm bands.
par.,	paratroch.
par.v.lft.,	left ventral paratrochal cell.
par.v.rt.,	right " " "
par.d.lft.,	left dorsal " "
par.d.rt.,	right " " "
proc.,	proctodæum, or anlage of same.
p.g.,	polar globules.
prot.,	prototroch.
som.pl.,	somatic plate.
stomod.,	stomodæum.

* The nomenclature is that of Wilson[1] somewhat modified, p. 230.

The blue tint is used to distinguish the "cross" and the somatic plate; brown, the prototroch; light red, the mesoderm; the entoderm is stippled.

† Other abbreviations used but once are explained in the special reference to the figure. For substitution letters, ap^1, ap^4, etc., see table, p. 236.

DESCRIPTION OF PLATES.

All the figures have been drawn with the aid of the camera lucida. No attempt has been made in most cases to represent the cell structure; on the contrary, various schematic devices have been introduced, *i.e.*, colors, heavy or dotted lines, shaded nuclei, etc., to assist one in following the more important groups of cells, although they certainly detract from the faithfulness of the representations. I have endeavored to select figures which represent every cell division up to a late stage in *Amphitrite*. In the very late stages and in the figures of the eggs of other species I have, for reasons of economy, left out many intermediate drawings and indicated the origin of cells by arrows only. The size of the figures has been determined by convenience and does not represent the relative size of the eggs.

EXPLANATION OF PLATE X.

Amphitrite ornata Verrill.

FIG. 1. Unsegmented egg, viewed from animal pole, showing first cleavage spindle inclined to future sagittal plane of embryo (line joining *x* and *y*), and nearer one end of the egg; *l* and *r*, left and right; *x*, ventral, *y*, dorsal; *m*, egg membrane.

FIG. 2. The 2-cell stage, viewed from vegetative pole: *AB* and *CD*, first two blastomeres.

FIG. 3. Viewed from one end: shows the spindle of first two blastomeres inclined to meridian at an early period; *p.g.*, polar globules; *an.*, animal pole; *veg.*, vegetative pole.

FIG. 4. Division of first two blastomeres nearly completed, viewed from vegetative pole: *A*, *B*, *C*, and *D*, first four blastomeres, the anlagen, respectively, of the left, ventral, right, and dorsal quadrants.

FIG. 5. The 4-cell stage, viewed from animal pole: karyokinetic figures not all of same age; *I–I*, first cleavage furrow; *II–II*, second cleavage furrow.

FIG. 6. Side view: division of the four cells into eight.

FIG. 7. The 8-cell stage from below (vegetative pole): *a*, *b*, *c*, and *d*, the four upper cells which constitute the umbrellar or upper hemisphere; A_1, B_1, C_1, and D_1, four lower cells which constitute the subumbrellar or lower hemisphere; *cr.f.*, cross-furrow; *seg.cav.*, segmentation cavity.

FIG. 8. From animal pole: transition from 8-cell to 16-cell stage. The lowermost products of the division of the four uppermost cells are the primary trochoblasts $a^{1.1}$, $b^{1.1}$, $c^{1.1}$, and $d^{1.1}$.

FIG. 9. From lower pole: transition from 8 to 16-cell stage.

FIG. 10. From left side: transition from 8 to 16-cell stage.

FIG. 11. 16-cell stage from above: nearly all the cells contain karyokinetic spindles in early stages which indicate the direction of the next cleavage.

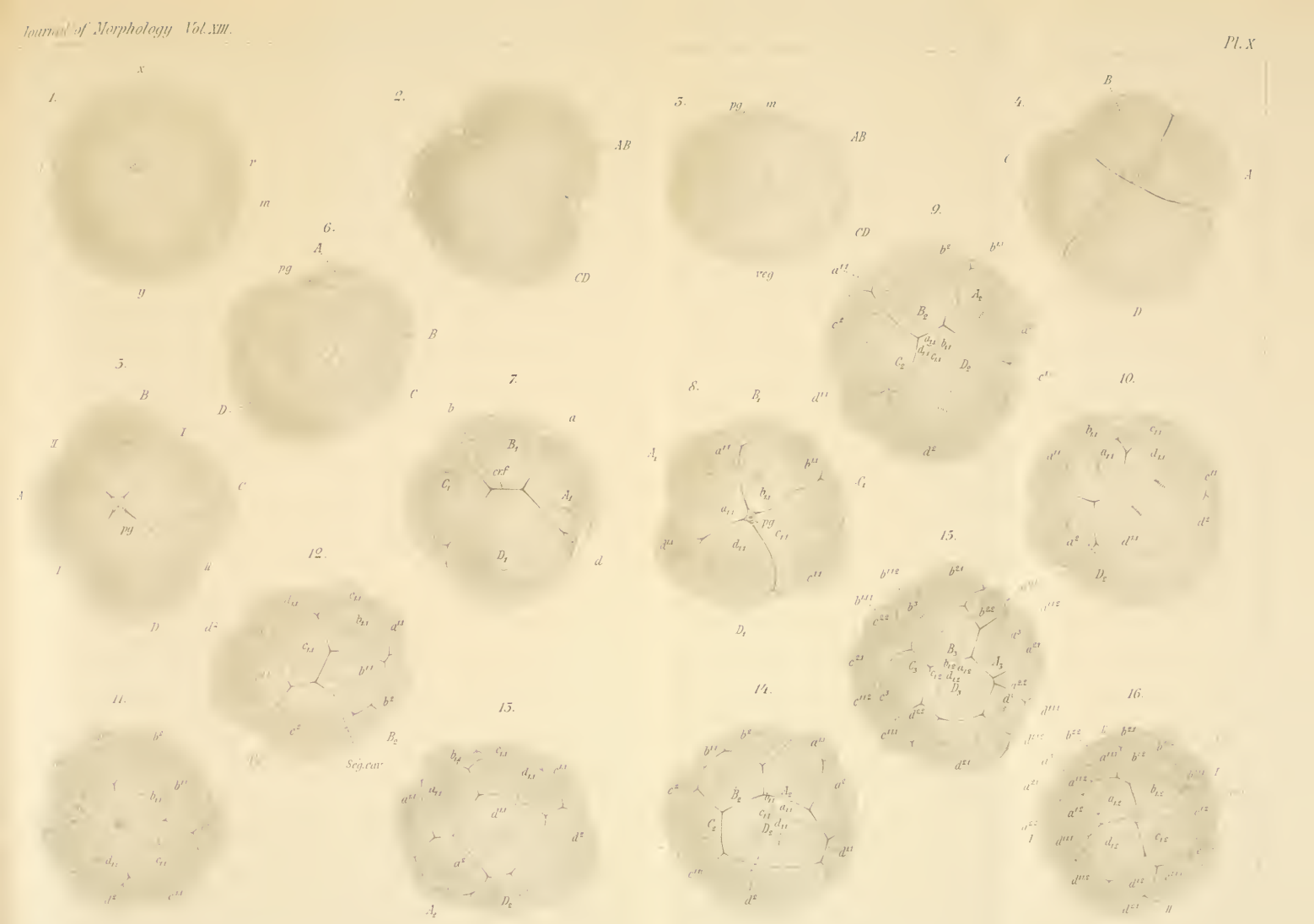
FIG. 12. Same egg as Fig. 11 from right side: *seg.cav.*, segmentation cavity.

FIG. 13. Same egg as Figs. 11 and 22 from left side: the spindles in $b_{1.1}$, $c^{1.1}$, d^2, and D_2 are seen from the end; the spindle in $d^{1.1}$ is in an abnormal position; it is in all other eggs I have examined parallel with that in $d_{1.1}$.

FIG. 14. Same egg from vegetative pole.

FIG. 15. From vegetative pole: later than last figure; transition to 32-cell stage.

FIG. 16. 32-cell stage from animal pole: the furrows drawn with heavy lines, *II–II*, indicate the direction of the second cleavage furrow; those in dotted lines, *I–I*, that of the first cleavage furrow.



Amphitrite

EXPLANATION OF PLATE XI.

Amphitrite.

Cross and somatic plate, blue; prototroch, brown; mesoderm, red; entoderm, stippled.

FIG. 17. From animal pole: the spindles in the four apical cells indicate the divisions resulting in the rosette; end view of spindles in the primary trochoblasts of second generation (colored brown).

FIG. 18. From animal pole: the primary trochoblasts of third generation, *i.e.*, the cells of the primary prototroch (brown), have developed cilia; apical rosette, $a_{1.3}$–$d_{1.3}$; parent cells of cross in blue; trochophore now rotates in direction indicated by arrow, *ar*.

FIG. 19. From vegetative pole, about same age as Fig. 18: primary mesoblast cell *M*, or d^4, being formed (colored red); somatic-plate cells in blue; primary prototroch in brown as before.

FIG. 20. Same view: mesoderm cell fully formed; superficial position of *D*. The two figures show an unusual time variation, the division producing d^4 (= *M*) being tardy in Fig. 19; other cells colored as before.

FIG. 21. Same egg as Fig. 20 from right side: color as in previous figures; cross and somatic plate, blue, etc.

FIG. 22. Same egg from left side.

FIG. 23. From vegetative pole, somewhat later: entoderm plate stippled; only posterior prototrochal cells visible; ciliated.

FIG. 24. Like Fig. 18, somewhat later stage.

FIG. 25. From left and below: same egg as Fig. 23.

FIG. 26. From right side and below: same egg as Figs. 23 and 25.

FIG. 27. From left side, about same stage as last figure: shows ciliation of ap^1 (= $a^{1.1.1.1}$), which in *Nereis* is non-ciliated (Wilson).

FIG. 28. Same egg as Fig. 27 from ventral side.

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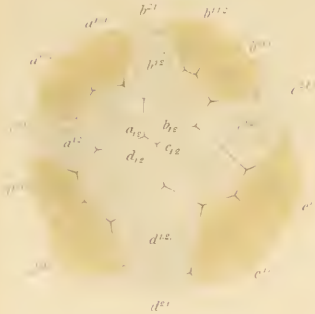

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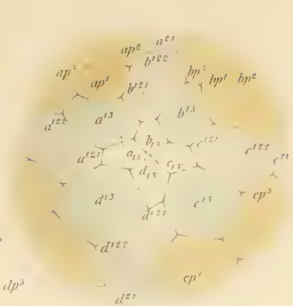

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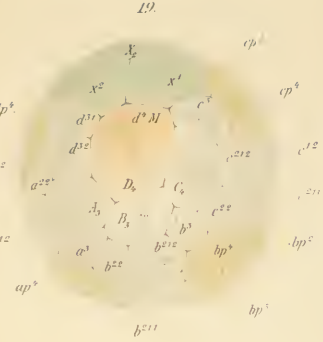

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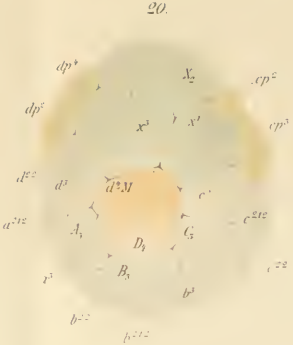

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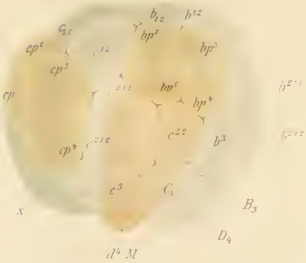

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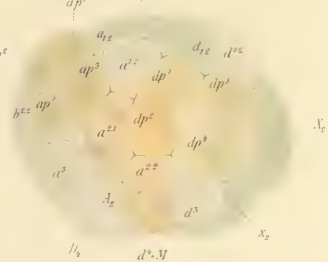

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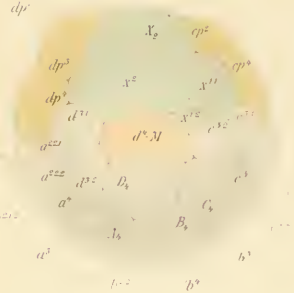

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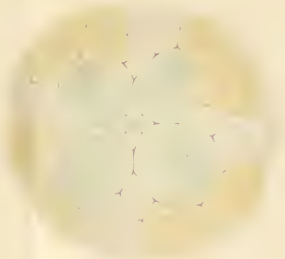

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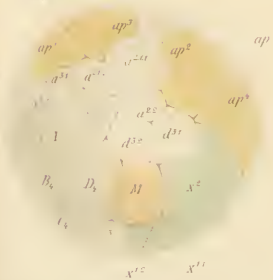

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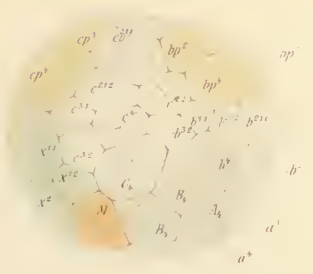

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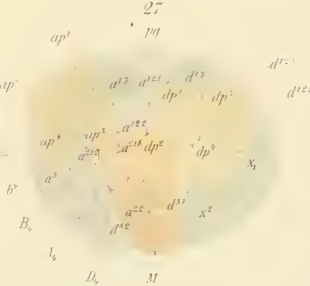

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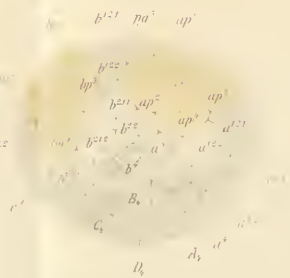


Amphitrite.

EXPLANATION OF PLATE XII.

Amphitrite.

FIG. 29. From animal (apical) pole: cross, blue; rosette in middle; mesoderm cells (red) and somatic-plate cells show through.

FIG. 30. From animal pole, somewhat later: rosette cells dividing; $d^{1.2.2}$ divided, its lower products indicated by shaded nucleus; mesoderm and somatic-plate cells as before.

FIG. 31. Same view: further divisions of cross cells; rosette left out.

FIG. 32. Same view: rosette divided; *gl.l.* and *gl.r.*, left and right-dorsal mucous glands of umbrella, corresponding to "head-kidneys" in *Nereis* (Wilson); *g* and *g*, corresponding cells in ventral arms of cross; $d^{1.2.2.2}$ about to divide (cf. previous Figs. 31, 32); *gl.l.*, *gl.r.*, mucous glands.

FIG. 33. Same view: further divisions of the cross cells (rosette not figured), *g* and *g* dividing; compare Figs. 31, 32 *gl.l.*, *gl.r.* mucous glands.

FIG. 34. Same view: dorsal part of umbrella; the lower daughter cells of $d^{1.2.2.2}$ (Fig. 32) dividing; the three products are l^1, l^2, and l^3; *gl.l.*, *gl.r.*, mucous glands.

FIG. 35. From animal pole, somewhat dorsal: $d^{1.2.2}$ dividing to form the important landmark (*); $d^{1.2.1}$ divided; x^3 dividing.

FIG. 36. Nearly same view as Fig. 35, later stage: *gl.l.*, *gl.r.*, mucous glands.

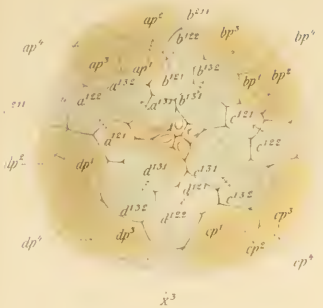
FIG. 37. Same view, somewhat later, showing left mucous gland cell, *gl.l.*, nearly covered by surrounding cells.

FIG. 38. Lower hemisphere, about the same stage as Fig. 29: somatic plate, blue; mesoderm *M* and *M*, red; entoderm, stippled.

FIG. 39. Dorsal view, little later than Fig. 38.

FIG. 40. Lower (subumbrellar) hemisphere: shows secondary trochoblasts cpc^5, p^6, cp^7; ap^5, ap^6, ap^7; bp^5, bp^6, bp^7.

29.



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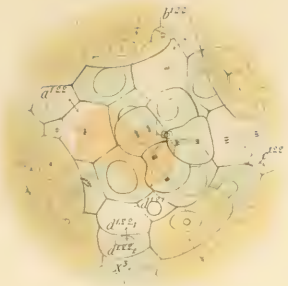

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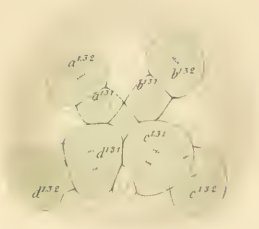

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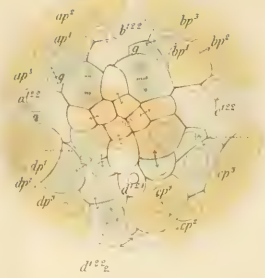

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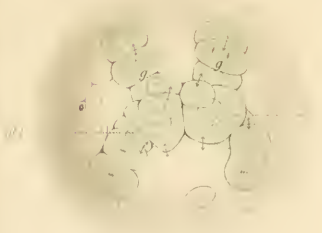

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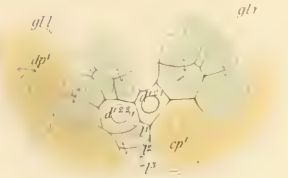

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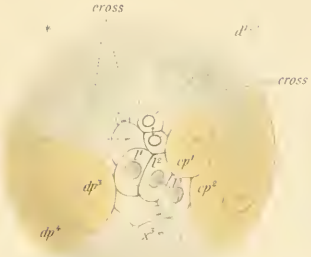

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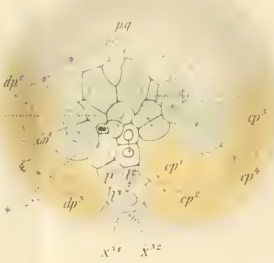

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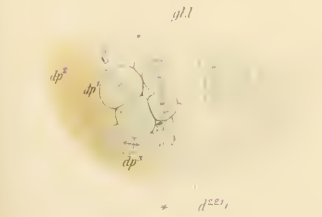

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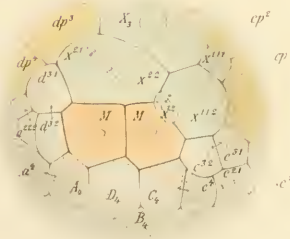

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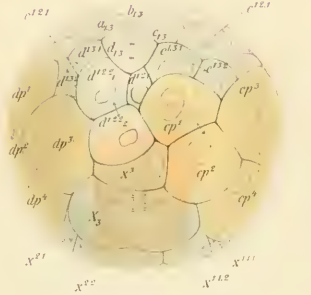

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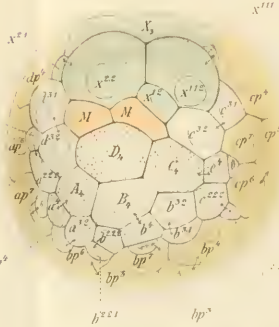


Amphitrite.

EXPLANATION OF PLATE XIII.

Amphitrite.

Prototroch, brown; somatic plate, blue; mesoderm, red; entoderm, stippled; the groups of somatic-plate cells descended from X_3, x^2, and x^1 separated by heavy lines.

FIG. 41. Lower hemisphere: mesoderm cells beginning to sink below the surface.

FIG. 42. Same view: mesoderm cells *M* and *M* partly covered and ready to divide and give rise to the minute cells seen in Fig. 46, *m* and *m*.

FIG. 43. Similar view, somewhat to left side.

FIG. 44. Same view as last figure and about the same age. The two figures show the variations in *time* of division.

FIG. 45. Dorsal view, showing the dorsal interruption in the prototroch.

FIG. 46. Lower hemisphere, dorsal: cells of entoderm plate dividing for the last time on the surface; ends of mesoderm cells still seen at the surface; *m* and *m*, small cells in segmentation cavity arising by division of the original mesoderm cells *M* and *M* (cf. Figs. 42 and 44).

FIG. 47. Lower hemisphere: definitive entoderm plate; mesoderm entirely within the segmentation cavity.

FIG. 48. Dorsal view: division of x^3.

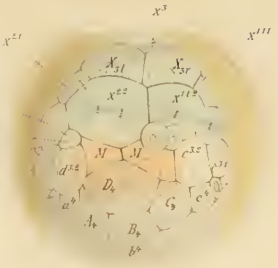
FIG. 49. Lower hemisphere, dorsal.

FIG. 50. Lower hemisphere, dorsal: mesoderm cells below the surface; the mesodermal teloblasts ready to divide the second time.

FIG. 51. Dorsal view, showing interruption of prototroch.

FIG. 52. Lower hemisphere: each mesoblast band composed of three cells.

41



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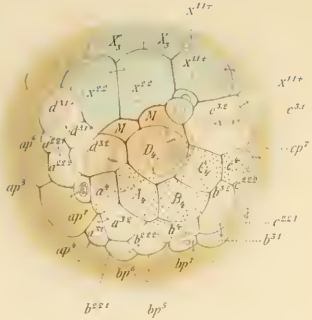

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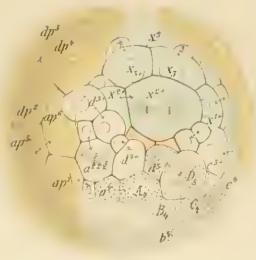

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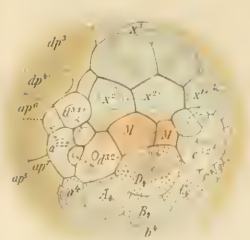

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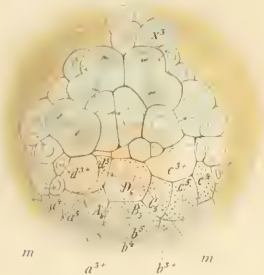

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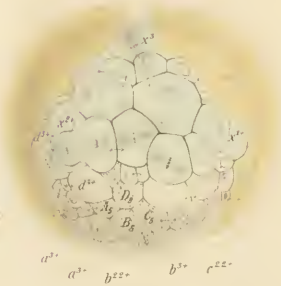

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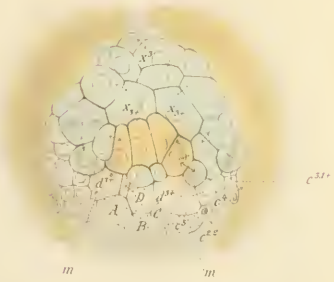

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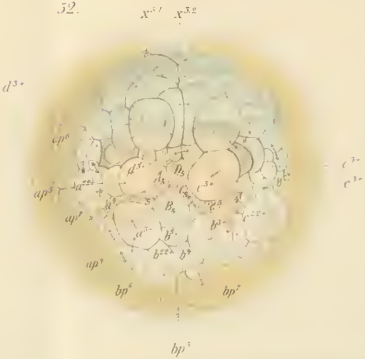


Amphitrite

EXPLANATION OF PLATE XIV.

Amphitrite.

Prototroch and *paratroch* brown; somatic plate, blue; mesoderm, red; entoderm, stippled.

FIG. 53. Lower hemisphere, dorsal: *par.v.lft.*, left-ventral paratrochal cell; *par.v.rt.*, right-ventral paratrochal cell; the two cells between them give rise after one division (cf. next figure) to the dorsal paratrochal cells.

FIG. 54. Same view: last division of paratrochal cells; *par.d.lft.*, left-*dorsal* paratrochal cell; *par.v.lft.* and *par.v.rt.*, left and right-ventral paratrochal cells.

FIG. 55. Same view, later: paratroch completely differentiated; *par.d.rt.*, right-*dorsal* paratrochal cell; others as in Fig. 54.

FIG. 56. Lower hemisphere: beginning of concrescence of somatic-plate cells from either side ventral to the paratroch; *proc.*, area of future proctodæum.

FIG. 57. Same view, later: further concrescence of somatic plate: letters x^2, $d^{3.1}$, etc., indicate from what cells the respective areas are derived.

FIG. 58. Dorsal view of stage like Fig. 56: interruption of prototroch; cells with shaded nuclei; 1^1, 1^2, 1^3, etc., descended from $d^{1.2.2}$ (cf. Fig. 30).

FIG. 59. Dorsal view: *gl.* and *gl.*, dorsal umbrellar mucous glands (cf. Figs. 32, 33, etc.); mesoderm bands, *mes.*, composed of six cells each; interruption in prototroch nearly completed; other letters as before.

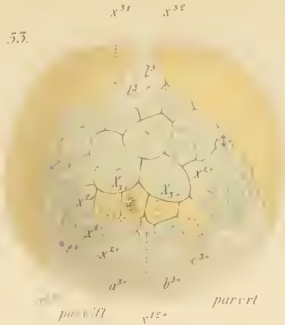
FIG. 60. Dorsal view: prototroch interruption obliterated by concrescence of prototroch cells.

FIG. 61. Right side: surface view of prototroch; the rest in optical section, showing entoderm cells, *ent.*, somatic plate, *som.pl.*; mesoderm band — three cells, teloblast dividing, *mes.*; paratroch cells, *par.d.rt.*, *par.v.rt.*, etc.

FIG. 62. Left side: same egg as Fig. 59; entoderm and mesoderm bands in the interior; letters as before.

FIG. 63. Right side, later larva, showing prototroch, paratroch, stomodæum (*stomod.*), etc.

FIG. 64. Optical section from left side: *prob.*, problematic bodies; *gl.l.*, left-dorsal umbrellar mucous gland (cf. Figs. 30 and 59); *gl.*, ventral umbrellar mucous glands; *duct*, duct of gland; *ext.op.*, opening of duct to exterior; *vac.*, vacuoles in cells of prototroch; *stomod.*, stomodæum; *muc.*, mucous glands of subumbrella; *n*, groove separating head segment from first trunk segment; *par.v.lft.* and *par.d.lft.*, ventral and dorsal paratroch cells of the left side; *proc.*, proctodæum. For later stages see text Figs. XI–XVIII.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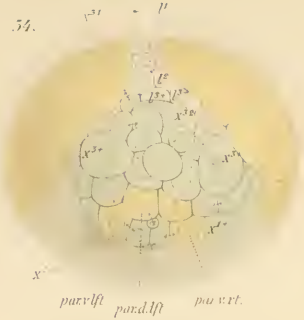
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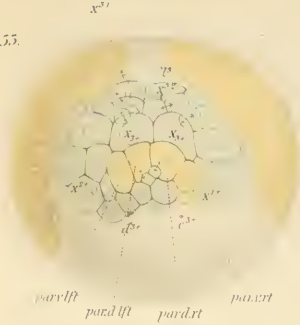
pard.lft
pa v.rt.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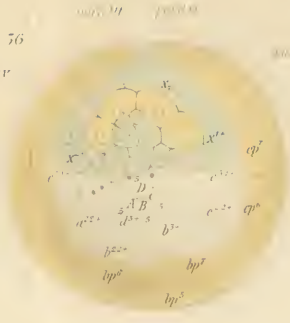
pard.rt
pav.rt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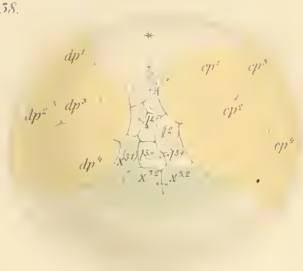
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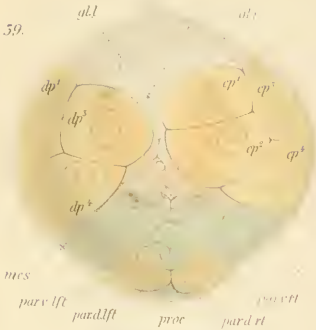
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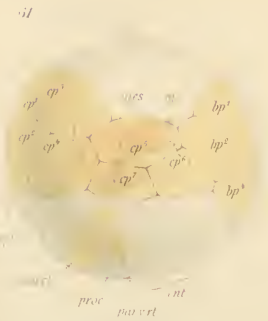
60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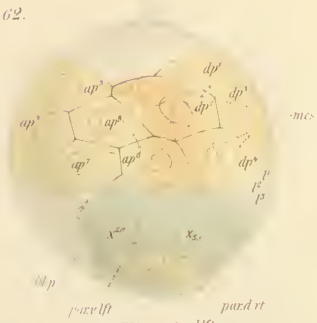
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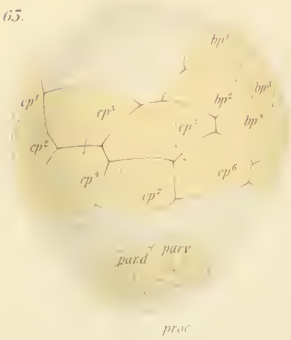
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pard
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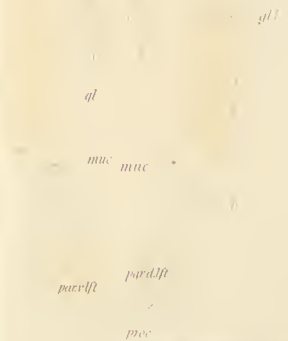
64
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muc
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Amphitrite

EXPLANATION OF PLATE XV.

Clymenella torquata Verrill.

FIG. 65. From animal pole (nearly) : 2-cell stage.

FIG. 66. Same view : 4-cell stage.

FIG. 67. From vegetative pole : 8-cell stage.

FIG. 68. 16-cell stage from animal pole : from life.

FIG. 69. Same view : thirty-two to sixty-four cells; formation of "apical rosette."

FIG. 70. Same view : primary prototroch complete

FIG. 71. Same view : rosette cells dividing.

FIG. 72. Same view : two of the rosette cells have divided; *gl.l.* and *gl.rt.* correspond to mucous gland cells in *Amphitrite*, "head-kidneys" in *Nereis*

FIG. 73. Lower hemisphere : 32-cell stage; from life.

FIG. 74. Same view: 32 to 64-cell stage; formation of mesoderm cell $M = d^4$.

FIG. 75. Right side : same stage.

FIG. 76. Left side : about the same stage.

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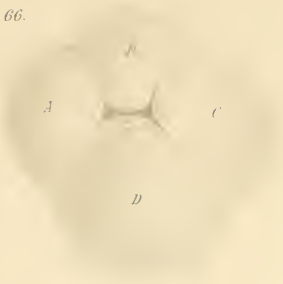

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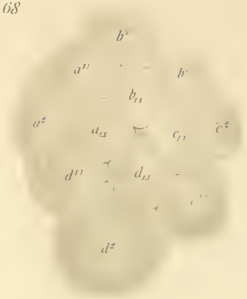

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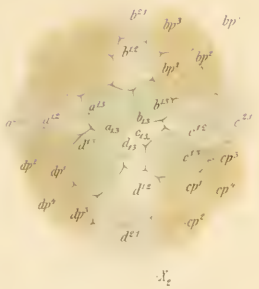

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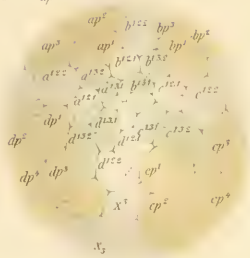

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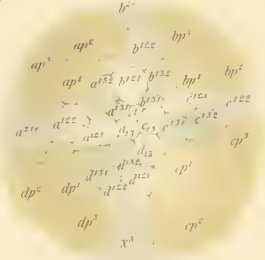

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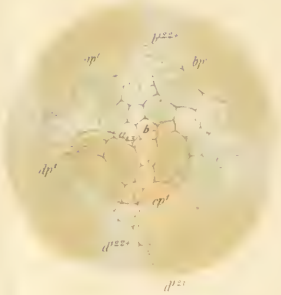

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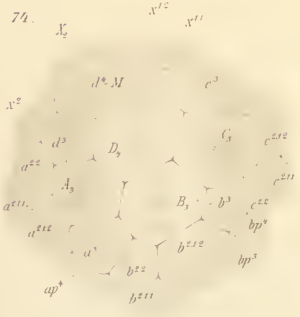

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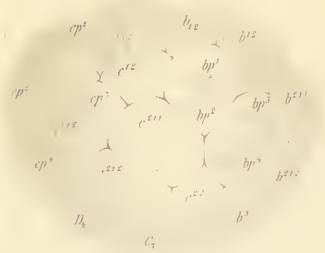

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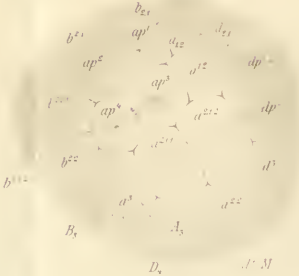

Clymenella.

EXPLANATION OF PLATE XVI.

Clymenella.

FIG. 77. Left side: later than last figures; first division of secondary trochoblasts.

FIG. 78. Lower hemisphere: about the 64-cell stage.

FIG. 79. Dorsal view: about the same stage.

FIG. 80. Left side: after 64-cell stage; formation of secondary prototrochal cells ap^5, ap^6, ap^7.

FIG. 81. Right side: same egg as Fig. 77.

FIG. 82. Lower hemisphere: same stage; mesoderm ready to divide.

FIG. 83. Same view, a little later.

FIG. 84. Lower hemisphere, later: last surface division of entoderm-plate cells — mesoderm divided.

FIG. 85. Same view, later.

FIG. 86. Same view: mesoderm cells getting ready to divide again; vacuoles in three entoderm cells, A_5, B_5, and C_5, indicated by faint lines; also the outlines of X_3 and X_3; entoderm plate complete; prototroch complete.

FIG. 87. Same view, little later stage.

FIG. 88. Lower hemisphere, somewhat dorsal: mesoderm cell divided; *m* and *m* correspond to the very minute cells in *Amphitrite* (cf. Fig. 50, *m* and *m*).

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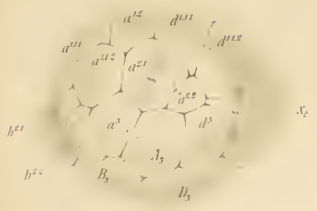

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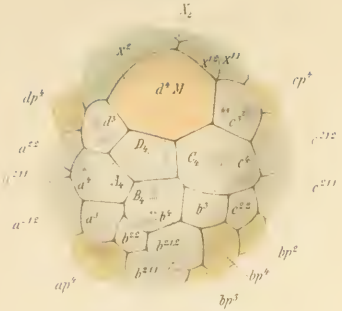

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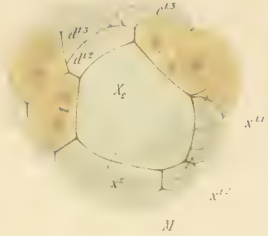

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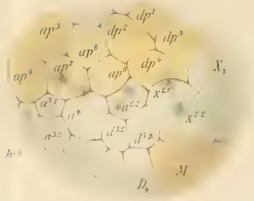

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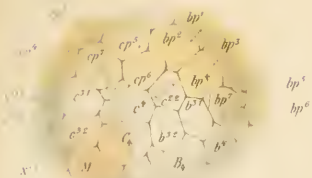

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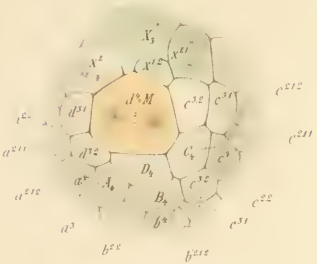

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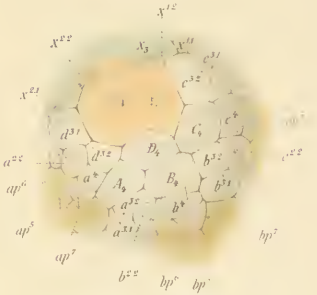

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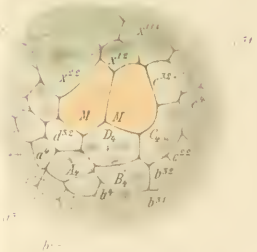

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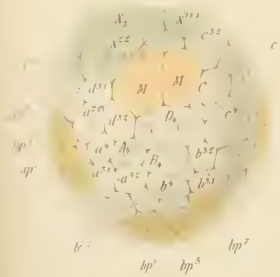

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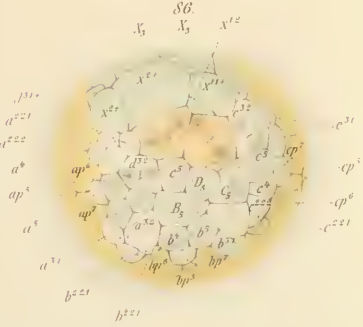

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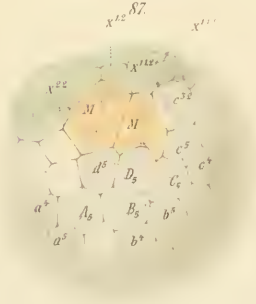


88.

EXPLANATION OF PLATE XVII.

Lepidonotus sp.

FIG. 89. Side view, showing that the first furrow sinks in at the animal and vegetative pole at the same time; *mem.*, egg membrane; solid line 1, outline at 11.27 P.M.; 2, outline of furrow at 11.28; 3, at 11.29; 4, at 11.31 P.M.; *p.g.*, polar globules.

FIG. 90. From animal pole: transition from 4 to 8-cell stage; cross-furrows at right angles to each other.

FIG. 91. From animal pole: 8-cell stage from life.

FIG. 92. Side view: 16 to 32-cell stage (cf. *Amphitrite*, Figs. 12 and 21, Pls. X, XI).

FIG. 93. From animal pole: thirty-two to sixty-four cells (cf. *Amphitrite*, Fig. 17); *pg.*, polar globules *inside* the apical cells.

FIG. 94. Lower (vegetative) hemisphere of same egg.

FIG. 95. Similar stage from vegetative pole: transparent; the cross and rosette cells (animal pole) in dotted lines.

FIG. 96. Similar stage from side, showing segmentation cavity.

FIG. 97. Side view, later: trochophore begins to swim; shaded cells are prototrochal (cf. Figs. 27, 28, *Amphitrite*, 77 and 80, *Clymenella*).

FIG. 98. Actual section of stage like Fig. 96 shows polar globules inside of apical cells, *p.g.*; segmentation cavity, *seg.cav.*

FIG. 99. Same egg as Fig. 96, from animal pole. Arrow indicates direction in which the trochophore rotates.

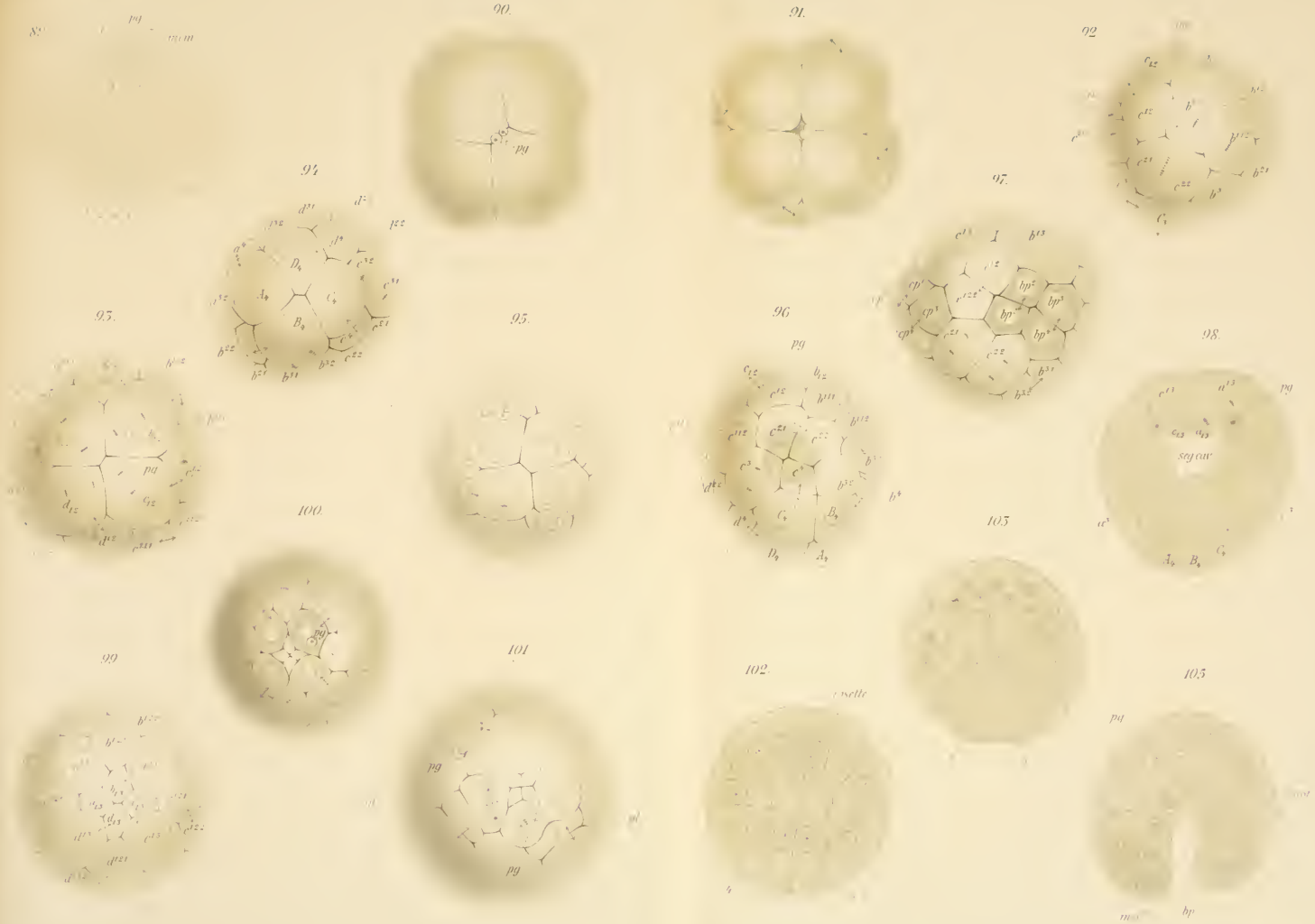
FIG. 100. Later stage: cross shaded, two cells in each arm (cf. Fig. 29); *p.g*, one polar globule, — the other within segmentation cavity.

FIG. 101. From animal pole: rosette and part of cross; three cells in some of the arms of cross, probably in all; *gl.*, cell corresponding to mucous gland in *Amphitrite* (cf. Fig. 32).

FIG. 102. Optical vertical section: the nuclei of the eight elongated vegetative cells are migrating inward. The cells 4, 4, are a^4, b^4, c^4, or d^4.

FIG. 103. Actual section, vertical, somewhat later.

FIG. 104. Optical section, gastrula: *p.g.*, polar globule; *prot.*, prototroch; *mes.*, mesoderm cell probably; *bp.*, blastopore.



Lepidonotus

EXPLANATION OF PLATE XVIII.

Scolecolepis viridis Verrill.

FIG. 105. From life, side view : first polar globule, *p.g.*; *m*, egg membrane.

FIG. 106. From left: 2-cell stage, vegetative pole. Precocious formation of cross-furrow.

FIG. 107. From life : three cells, seen from animal pole.

FIG. 108. From life : 4-cell stage, seen from animal pole.

FIG. 109. From life : animal pole and side.

FIG. 110. From life : 16-cell stage.

FIG. 111. Vegetative pole : same stage.

FIG. 112. 16-cell stage : cells tinted brown correspond to the *primary trochoblasts* of *Amphitrite* and *Clymenella*.

FIG. 113. Lower hemisphere, after division of cells seen in Fig. 110 : from life.

FIG. 114. From animal pole : cells tinted brown as before; cells $a^{2.1}$, $b^{2.1}$, and $c^{2.1}$ correspond to the *secondary trochoblasts* of *Amphitrite* and *Clymenella*.

FIG. 115. Trochophore with three trunk segments; *eye*, eye-spot; *prot.*, prototroch ; *par.*, paratroch ; *m.*, mouth ; *I*, *II*, *III*, trunk segments.

FIG. 116. Optical section, side: *muc.*, mucous gland; *prot.*, prototrochal cells ; *ent.*, entoderm ; *mes.*, mesoderm.

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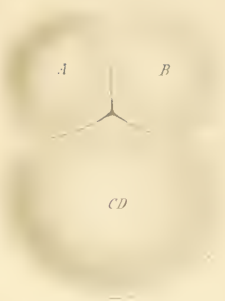

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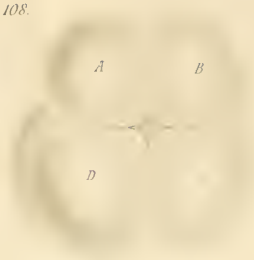

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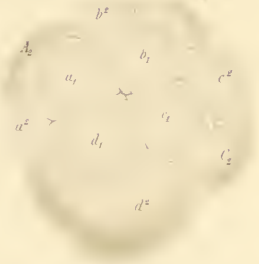

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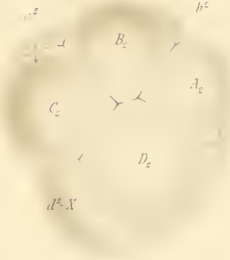

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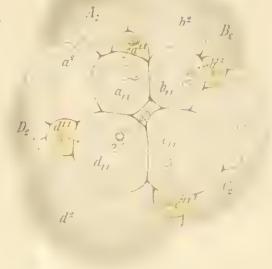

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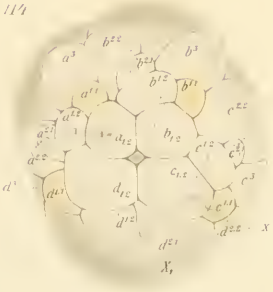

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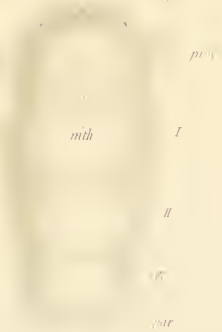


116.

Scolecolepis

EXPLANATION OF PLATE XIX.

Chætopterus pergamentaceus Cuvier.

FIG. 117. Side view, first cleavage; the yolk-lobe, *y*, in a very early stage.

FIG. 118. Same view, a little later.

FIG. 119. Same view, later still.

FIG. 120. Same view: yolk-lobe almost completely constricted off; nuclei of two cells nearly reconstituted.

FIG. 121. Same view: nuclei in resting stage; yolk-lobe has been resorbed.

FIG. 122. From animal pole: transition from eight to sixteen cells.

FIG. 123. From animal pole: 16-cell stage.

FIG. 124. Same view: 32-cell stage.

FIG. 125. Left side: just after 32-cell stage.

FIG. 126. From animal pole: nearly a 64-cell stage.

FIG. 127. From right side: same stage as last figure.

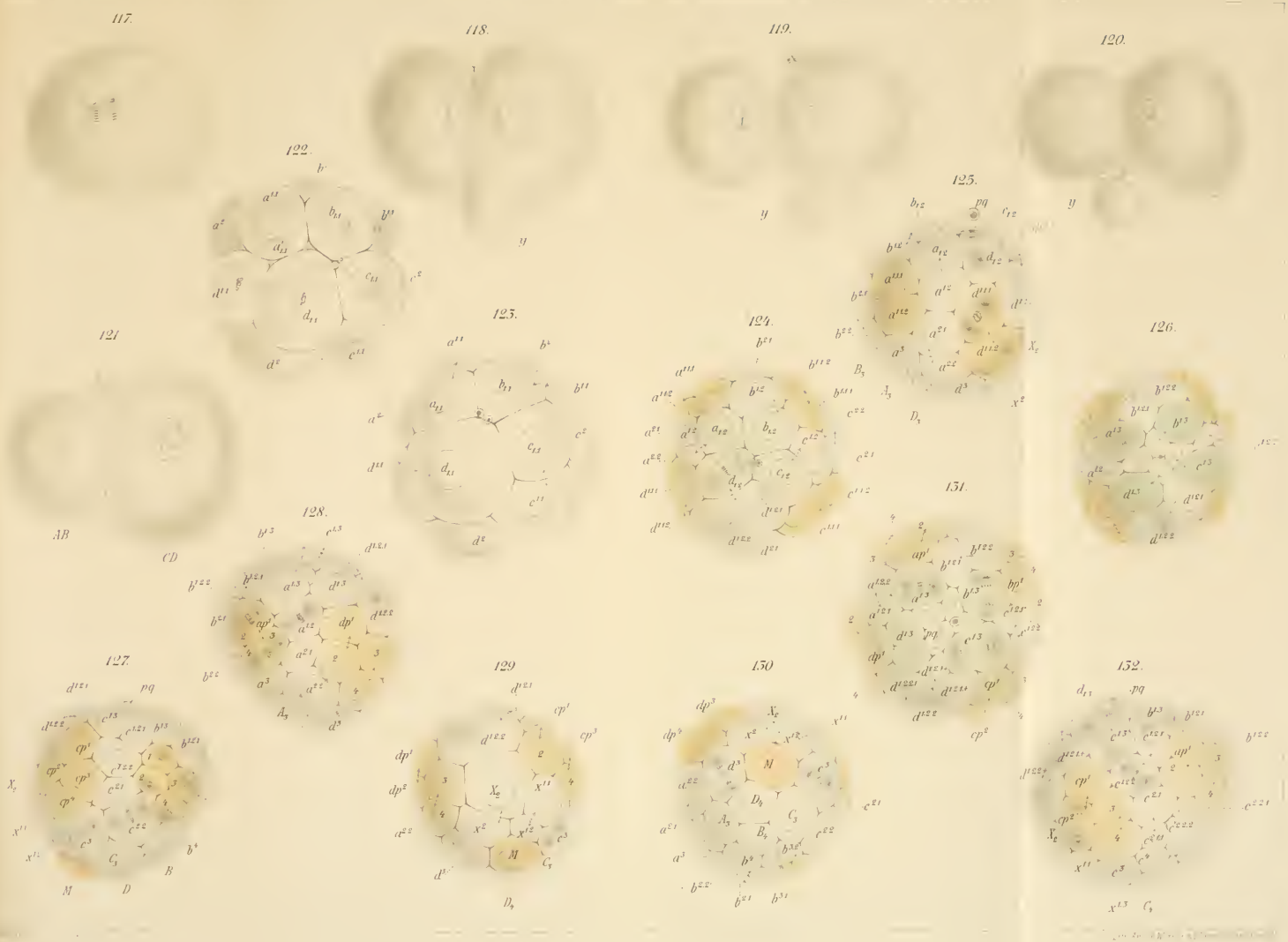
FIG. 128. From left side: same stage.

FIG. 129. Dorsal view: same stage.

FIG. 130. Lower pole: same stage.

FIG. 131. From vegetative pole: cross cells dividing *obliquely;* one of the polar globules within the rosette cell, *p.g.*

FIG. 132. Right side of same egg: *p.g.*, polar globule; one prototrochal cell, cp^2, *dividing again.*



Chætopterus.

Volume XIII. *May, 1897.* *Number* 3.

JOURNAL

OF

MORPHOLOGY.

ON THE STRUCTURE OF THE DISCODRILID NEPHRIDIUM.[1]

By J. PERCY MOORE.

1. *Number and Position of the Nephridia.*

Although no less than six[2] accounts of the discodrilid nephridium are already extant, these fail to furnish us with the information necessary to a careful comparison of the organ with its homologue in other annelids. Five papers dealing with the general anatomy of Branchiobdella parasita and its varieties contain accounts of the excretory organs, but of these only one, that of Lemoine (25), was based upon investigations conducted with a knowledge of modern methods of research. Though in some respects more complete than its predecessors, Lemoine's description adds little of importance to the earlier accounts,

[1] Contributions from the Zoölogical Laboratory of the University of Pennsylvania. No. 5.

[2] Voinov has recently contributed, after the present paper was in the hands of the publishers, a more complete and accurate account of the nephridium of Branchiobdella parasita. I have seen only the preliminary account (Comptes Rendus Acad. Sci., Paris, CXXII (1896), 1069–1071) with which in the main my results agree. This author is to be credited with the first correct description of the relations of the several regions of the nephridium. The "red gland" is identified as a folded canal; and excretion by fragmentation of the peritoneal corpuscles described.

with which it is in substantial agreement. The sixth and latest paper (**28**) mentions a few novel facts observed in Bdellodrilus illuminatus, but is very incomplete. A new investigation being therefore desirable, I am led to present the results of some recent studies.

Odier and Henle, writing respectively in 1823 and 1835, noticed that the nephridia were reduced in number to two pairs, a fact which has since been repeatedly verified for Branchiobdella parasita, and in the American discodrilids Bdellodrilus illuminatus, B. philadelphicus, B. manus, Branchiobdella instabilia, B. pulcherrima, Pterodrilus distichus, and P. alcicornus, as well as in several undescribed species. Consequently two pairs of definitive nephridia may safely be stated as characteristic of the family. This is a remarkable peculiarity in an annelid, in which group the metameric repetition of the nephridia is so general as to have gained for them the name of segmental organs, first proposed by Williams (**37**). Although the nephridia are not infrequently absent from a few of the anterior segments, and in Uncinais litoralis (**12**), and perhaps a few other naids, entirely wanting, no case exactly similar to the discodrilids is known. When, however, we consider the cephalization of the anterior somites, with the nearly complete obliteration of their coelom by encroaching muscle fibres and glands, the parallel concentration to form the sucker and its support at the posterior end, the shortening of the discodrilid body until it represents only the anterior (genital and pre-genital) segments, plus the anal and preanal segment of, for example, an enchytraeid; and when we consider the further fact that nephridia are absent from the genital segments of the latter, the important factors in an explanation of the discodrilid condition are in our possession.

This does not, however, explain why with four possible nephridia-bearing somites anterior to the genital region, only one pair of pre-genital excretory organs should persist in the adult worm. In this connection the following associated facts may be considered as possible, but not necessarily complete explanations, *viz.*, the large size of the uninary calculi and granules, necessitating a tube of considerable calibre to permit their

ready passage, the large heart with its prominent loop in the first and second somites, the enlargement of the alimentary canal in the fourth somite, the very narrow septa, and the active muscular movements of the worms. These conditions would evidently render a spreading longitudinal arrangement of the tubules an advantageous one, inasmuch as it would permit a more ready adjustment of the organs to the animal's movements. Sections of embryos indicate the presence of provisional nephridia in certain anterior somites, as has been shown to be the case in certain Oligochaeta and Hirudinea.

Individual nephridia are highly developed and conspicuous, the principal portion of each one consisting, as was originally described by Henle (21), of four distinct tubules arranged in two long loops, and passing into an opaque granular mass, through which previous writers have failed to trace them. In the more transparent species, and especially in the colorless-blooded Branchiobdella instabilia and pulcherrima, these granular masses may be seen with the naked eye as conspicuous, dark, and opaque spots, lying within the coelom by the side of the alimentary canal, one each in the second and third somites, and a pair symmetrically placed in the eighth somite.[1]

Connected with the masses are the nephridial funnels and efferent ducts, as well as the tubule loops, so that an understanding of their structure is necessary to a knowledge of the relations of the latter.

As indicated above, and this is true for all known species, the anterior nephridia are not symmetrical, the opaque mass of one lying anterior to that of the other. This has been observed by all writers on B. parasita, but it is noticeable in their descriptions and figures that they fail to agree as to whether the right or left one is the more anterior. Lemoine finally states that the species is variable, some individuals having the right, some the left nephridium in advance. This is the case in the three American species (Bdellodrilus illuminatus, B. philadelphicus,

[1] The European writers have variously enumerated the somites, some counting the "head" as one, and each two of the body rings as one; others each ring of head and body as a somite. Inasmuch as the constitution of the "head" is still a matter of disagreement, I enumerate only post-cephalic somites, each consisting of two annuli.

and Branchiobdella instabilia) which were examined with reference to this point. It is of interest that certain localities seem to furnish individuals predominantly of one kind, a fact which will be further developed in another connection.

In those species which have well-developed inter-segmental septa, as is the case in Bdellodrilus philadelphicus, Branchiobdella instabilia, pulcherrima, and parasita, the opaque masses are strictly confined to their proper somites. On the other hand, those of B. illuminatus, in which the septa of the segments anterior to the genital region are imperfect or altogether aborted, are more variable in position. The anterior one may lie partly or wholly within the third somite, and the posterior partly or wholly within the fourth, or both may lie — but this is a rare condition — symmetrically within the third.[1] In this species the greater number of individuals examined up to the present time had the anterior nephridium to the left, the posterior to the right of the intestine.

The general arrangement of the tubules can best be seen when living worms are examined under a low power. For convenience, let us suppose an example of B. instabilia in which the right nephridium is anterior — but the windings have practically the same form in all species. Three somewhat complexly looped tubules pass around the margin of the compact granular mass from the ventral to the dorsal anterior border, where they turn backward and are joined by a fourth, which appears from within the opaque mass. The four are now arranged in two pairs, of which one, composed of two of the tubules which arise from the ventral margin, forms a longer loop, and is twisted and folded about the other comparatively straight one. The four tubules, lying side by side, now leave the opaque mass at its posterior dorsal region, bend to the left, and extend transversely over the intestine, in more or less close contact with the septum $^{11}/_{12}$, to the left body-wall, to which they are anchored by their peritoneal investment. Turning sharply forward they extend in a loose, wavy loop to the anterior left angle of the same somite, and are here once more anchored to

[1] One example of the anterior opaque masses, both lying in the third somite, was observed in B. instabilia.

the body-wall. Again turning sharply, the longer loop being anterior, they cross the alimentary canal transversely, still on the dorsal side, to the right anterior angle of the segment, where the longer loop turns backward around the end of the shorter one, and the peritoneal covering extends as a sheet (with the apparent exception of B. illuminatus) to become continuous with the parietal peritoneum. At this point the two tubules of each pair become continuous with one another to form the two complete loops. The figure formed by the tubules of the anterior nephridium in the case described is seen to be a nearly complete quadrangle, extending around the four sides of the second somite (the anterior transverse leg frequently crowds the septum $^{I}/_{II}$ far forward), and terminating nearly at the point of apparent origin from the granular mass.

When the anterior nephridium lies on the left side, which is the more usual condition in B. illuminatus, the tubules are generally disposed in a different way. The tubule group first extends backwards from the anterior margin of the opaque mass to the septum $^{II}/_{III}$, then forward dorsally on the left side to the anterior septum, and transversely to the right, so that the tubule loops terminate at the same place as before. This terminal point is not, however, an absolutely fixed one, for rarely the case is found of a left anterior nephridium with the tubules arranged in quadrangular form, but reversed so that the loops begin and terminate on the left side. We find, then, two plans of disposal of the tubules — the quadrangular, occurring usually when the right one is anterior, and the zigzag, seen usually when the left one is anterior.

Comparing the published figures of nephridia of B. parasita, we find that Dorner (14) shows a left with the zigzag, while Lemoine figures a right with the quadrilateral, so that this species also appears to be liable to the same peculiar variation, the full significance of which is not now apparent, but which appears to be related to the position of the heart loop.

In the second nephridium, when on the left side, the tubules extend from the anterior border of the opaque mass along its ventral margin, pass through the neural arcade of the septum $^{III}/_{IV}$, then ventrally by the side of the alimentary canal to the

strong septum $^{IV}/_{V}$, in contact with which they arch upward over the intestine and cross to the right side, turning there ventralward, and becoming attached to the body-walls. When on the right side the tubules first pass dorsally over the opaque mass, and transversely from right to left.

The funnel and efferent duct are connected with the opaque mass at nearly the same point, which is about opposite to the place of origin of the tubule group. Keferstein (24) in Branchiobdella, and the writer in Bdellodrilus, have traced the efferent duct to its communication with the tubule loops. After leaving the opaque mass the efferent ducts become conspicuous tubules which pass transversely around the major annulus of the third somite to open by separate dorsal pores (Branchiobdella), or by a common median dorsal vesicle and pore (Bdellodrilus, Pterodrilus).

The post-genital pair of nephridia are confined to one segment, already largely occupied by the muscles of the sucker; they are consequently less conspicuous than the anterior. They lie in the eighth somite symmetrically[1] on each side of the intestine, and in B. instabilia extend into the flange-like lateral flattenings of this region. As Henle (21), Dorner (14), and Lemoine (25) have already mentioned, the posterior nephridia, although obviously constructed on the same plan, are much more irregularly arranged than the anterior, which results from the necessity of the tubules to accommodate themselves to the muscle fibres which traverse and divide the coelom. The tubules are smaller and in many ways more difficult to study. The granular mass is rather small and lies at about the middle of the segment. The tubules are arranged in a U-shape around it, the open end of the U forward, and away from the granular mass. The outer limb is the shorter and the more complex. The six tubules which it contains may be traced as three which arise from the mass, extend forward on its outer margin, and return to pass around it to the inner side, continuing there as the inner lobe, which receives a fourth tubule — the four being then grouped into two loops which reach forward to the septum $^{VII}/_{VIII}$. On comparison with an anterior nephridium it will be

[1] Lemoine states that in B. parasita the left is slightly in advance.

seen that the inner lobe, with its two loops reaching to the septum $^{VII}/_{VIII}$ corresponds to the long lobe of four tubules, while the outer corresponds to the smaller lobe of three tubules doubled on itself. The two are then bent around the granular mass and more compacted. The pairing of the tubules is also less distinct, as they often separate and pursue more or less independent and often highly irregular courses.

European authors have described the tubules as ciliated throughout, even the efferent duct being so described by Lemoine and figured by Dorner.[1] Such is not the case, however, in American species, in which the efferent duct, that portion of it at least which lies in the body-walls, is entirely without cilia. The same is true for the greater part of the short tubule loop, while in the longer loop the cilia are arranged at regular intervals in widely separated groups. The bewildering activity of the ciliary action in all parts of the organ as it lies folded within the body might well lead one, on superficial examination, to suppose that the ciliation was general; and doubtless it will be found that B. parasita does not differ from the American species, which approach the usual oligochaetous plan in the scattered arrangement of the ciliated tracts.

2. *Analysis of a Nephridium of Bdellodrilus illuminatus.*

The foregoing, in addition to describing the general arrangement of the nephridia in American forms, contains practically a summary of the morphological facts furnished by former writers. Before describing in detail the minuter structure of the nephridium, it will be well to proceed to its analysis, selecting as a type a species in which this is easily accomplished, and then to consider each region in turn.

Referring to the figure of Bdellodrilus illuminatus (Pl. XX, Fig. 1), and to the simplified diagram (Fig. 2), and beginning at the inner end (that most remote from the external pore), we find the ciliated funnel (*t*) containing the nephrostome (*nn*), and passing into a narrow tubular neck (*ts*), which is connected with the yellow granular mass, not highly opaque in this species.

[1] Voinov (l. c.) has corrected these statements, finding in B. parasita a distribution of the cilia similar to that here described.

This mass is seen in B. illuminatus to be in reality a complexly folded and beaded section of the tubule (*mp*, *ap'*) composed of swollen and highly granular nodules (*pn*) alternating with short narrow sections (*ct*). As the lumen breaks up within each of the nodules into canalar plexuses, we may designate this the plexus region, in which we distinguish a main plexus lobe (*mp*, *mp''*) and an accessory plexus lobe (*ap*, *ap'*), the latter of which is connected with the system of spreading tubules which constitute the most noteworthy part of the organ. The tubule arising from the external end of the plexus is the outermost of a group of three (*sl*, *sl*) which is closely associated with the accessory plexus lobe, and which will be denoted the small tubule lobe. Leaving this and passing directly away from the plexus region, the first tubule reaches to the farthest point to which the nephridium extends, then turning on itself forms a recurrent canal which returns to the starting-point, becoming there the middle tubule of the small lobe; but bending once more as the innermost tubule of this lobe, it retraces the course nearly to the farthest point reached, and then returns as a second recurrent tubule to the plexus region, but not this time into the smaller tubule lobe. Thus is constituted the large tubule lobe (*ll*, *ll*), composed of four tubules arranged in two loops which communicate at their proximal ends by means of a short connecting tubule (*cct*). Of the two loops the second one is the shorter (*st*, *st*), and may be said to constitute an axis, about which the other is arranged and beyond which it extends. The latter is therefore much the longer loop (*lt*, *lt*).

From the proximal end of the recurrent limb of the shorter loop the tubule (*et*1, *et*2) proceeds along the axis of the main plexus lobe to the point of attachment of the funnel stalk, where it connects with the coelomic portion of the efferent duct (*et*2, *et*3), here thrown into a loop which embraces the neck of the funnel. Passing from this point to the body-wall, it perforates the longitudinal muscular layer, and proceeds dorsalwards in the intermuscular space to the common vesicle by which it opens with its fellow to the exterior (*et*3).

We have now traced the nephridium through all its parts, and have found that it is throughout a continuous tubule, in which

have been distinguished a number of successive regions, most of which will be found to present structural peculiarities. To recapitulate, these regions are the funnel and its stalk, the plexus region, with its alternating plexus nodules and simple tubules, its main and accessory lobes, the outer and inner (or recurrent) limbs of the longer tubule loop, the connecting tubule, the outer and inner limbs of the shorter tubule loop, the efferent duct with its connecting, coelomic, and intermuscular sections, leading to a terminal vesicle. Were this entire tubule extended it would have in a moderate-sized worm (3 mm. long) an estimated length of 7 mm., or more than twice the length of the entire animal. This would be distributed among the several regions as follows: funnel and stalk, .1 mm.; plexus region, 2 mm.; longer tubule (complete), 2 mm.; connecting tubule, .4 mm.; shorter tubule loop, 1 mm.; efferent duct, connecting section, .5 mm.; coelomic, .4 mm.; intermuscular, .6 mm. For its entire length the tubule presents a fairly uniform diameter, with, however, about twenty bead-like enlargements in the plexus region and a less conspicuous one uniting the coelomic and intermuscular efferent ducts. The tubule of the nephridium shown in Fig. 1, taken from a worm of 3½ mm. in length, measured nearly 9 mm. in the course of its windings.

3. *The Funnel.*

Each of the four nephridia is provided with an open ciliated funnel, which, although of large size, is, owing to its transparency, rather inconspicuous. Its position is similar in all of the species. That of the anterior nephridium is suspended by its slender stalk from the extreme postero-ventral margin of the plexus mass, and rather from its external than its mesial face. Its obliquely truncate nephrostomal end usually faces ventrally, internally, and posteriorly, and nearly touches the body-floor. Its position may, however, be slightly shifted. The funnel does not stand out freely, as represented in Dorner's figure (nor is its position in American forms as there shown), but its base and stalk are closely embraced by folds of the efferent duct, as shown for several species in Figs. 1, 6, 9, and 10, and in B.

instabilia scarcely more than its ciliated mouth appears. The second funnel has the same relation to its nephridium as the first, but owing to its reversed position hangs from the anterior ventral margin and faces forward. Fig. 11, which represents a horizontal section near the floor of the body of B. instabilia, shows the relation of the pre-genital funnels to the coelom. The first one (t') lies entirely within somite II, but very close to its posterior septum, where it is held by the efferent duct which perforates that septum. The second funnel (t'') is similarly held by its efferent duct close to the point where that duct enters the body-wall of somite III. In B. illuminatus the imperfect septa permit a greater range of movement to the funnels, and the anterior one is frequently found in somite III, which it always faces. Figs. 13, 14, and 15 show respectively the points where the funnels enter the plexuses of B. illuminatus, B. pulcherrima, and B. philadelphicus, all on the extreme ventral margins (*tp*, *t*, and *fs*). Fig. 1 also shows its relation to the plexus. The funnels of the posterior nephridia lie in lateral positions about the middle of somite VIII, and face posteriorly and mesially. Their relation to the plexus is the same as in the anterior nephridia (Pl. XXI, Fig. 12, *t*).

The funnels of the several species, although agreeing in essential structure, differ somewhat in form. That of Bdellodrilus illuminatus (Pl. XXI, Figs. 5, 6, 7) has somewhat the shape of a leg of mutton, its stalk being somewhat narrowed and constricted by the enclosing folds of the efferent tubule, the free portion decidedly enlarged and the nephrostomal end oblique and somewhat constricted. In B. philadelphicus (Fig. 8) the funnel is more or less irregularly pear-shaped, but also slightly flattened, oblique, and swollen. Branchiobdella instabilia and pulcherrima have very pretty symmetrical funnels of nearly the bell-jar shape described for B. parasita by Dorner, but here also somewhat flattened (Fig. 9). The nephrostomata also differ; that of B. illuminatus is small and elliptical, or sometimes slit-like, that of B. instabilia larger and rounded, and that of B. philadelphicus very conspicuous, and in the example figured (Fig. 8) as large as the funnel cavity, which is much wider than the nephrostome in B. illuminatus (Fig. 5).

In Bdellodrilus the nephrostomal lip is formed of only two marginal cells (Pl. XXI, Figs. 5, 7, 8 *mn*). These are not of equal thickness all around, but each is conspicuously swollen dorsally, and much thinner ventrally (Fig. 10[a]), thereby causing the obliquity of the terminal face and the excentric position of the nephrostome. In B. illuminatus the marginal cells are, during life, conspicuously roughened by rounded eminences which simulate cells and are richly ciliated. Branchiobdella instabilia and pulcherrima have three marginal cells, two of which are dorsal and swollen, the third ventral and much thinner. This latter may really be slightly displaced from the margin and correspond more nearly to the central cell of Lumbricus, etc. Fig. 9 is a dorsal view.

Longitudinal sections of the funnel of B. philadelphicus (Fig. 10) show a direct continuity (*pm*) of the outer boundaries of the marginal cells with the unmodified peritoneal layer which invests the remainder of the funnel and nephridium. This layer (*p*) is not distinctly cellular, but if one of the large nuclei which here and there occur (Pl. XXIII, Fig. 52) be near for comparison, as in the funnel figured, the resemblance to the nuclei of the marginal cells is very striking. The latter have the appearance and anatomical relations of greatly enlarged peritoneal cells. During life the protoplasm of these cells is transparent, with rather large and sparse granules. The nuclei are very transparent, of large size, and with a single prominent chromatin body. The whole outer and nephrostomal surfaces are covered with rather short cilia (Figs. 5 *et seq.*), which lash so vigorously that the funnel vibrates with the motion, as does a Vorticella when feeding. In sections stained with haematoxylin (Pl. XXI, Figs. 10, 10[a], 10[b]) the protoplasm appears very homogeneous, except in a zone superficial to the nucleus, which is more granular and more intensely stained.

The funnel proper is completed by a single additional cell, which in its optical characters, both when living and when stained, does not differ from the marginal cells. This I have called the central cell, but it differs from the central cell of the funnel of the earthworms (6) in being tubular. This cell (in Figs. 6, 7, 8, 10, and 10[b]) joins the marginal cells on the one

hand, the stalk cell on the other. Like the former it is thickened dorsally where the nucleus is situated, and is thinner ventrally (Figs. 10, 10[b]); like the latter it is tubular, and bears a longitudinal tract of cilia within its lumen. These cilia are very long, and arise from the dorsal wall contiguous to the nucleus. In B. illuminatus they all extend down the funnel (Fig. 6), while in the other species some of the upper ones pass outward through the nephrostome with the marginal cilia (Figs. 8, 9, and 10). They beat with a rapid wave-like motion, which here recurs much more frequently than in other parts of the nephridial tubule. The marginal cilia have a rotary motion which is very obvious at the nephrostome, and in the few favorrable views which I have had from right to left (Fig. 7).

The funnel stalk is a simple narrow tubule (Figs. 5, 6), exactly like the simple tubules of the plexus region, except that it is narrower and the course of its lumen sometimes more irregular, or rarely even spirally wound within the tubule (Figs. 1, 5, and 6).

4. *The Plexus Region.*

With regard to this region of the nephridium the accounts of Branchiobdella are very unsatisfactory. Odier (29) has called the massive portions of the nephridia the red glands. Henle (21) designates the region as the yellow granular body, and notes its glandular nature, and its enclosure of a tissue of twisted tubules. Keferstein (24) describes it as a yellow glandular mass containing a snarled group of tubules enclosed by granular pigmented cells. Dorner (14), more fully but somewhat uncertainly states that "the canal into which the funnel passes is immediately thrown into many closely lying folds united into a thick skein and covered by a yellow-brown pigment. This becomes more massive with the growth of the animal, and in the adult is visible to the naked eye as a brown spot; in young examples the separate windings are more distinctly perceived. . . . That the tubes present in the skein are parts of a single canal bending closely against and around itself cannot certainly be concluded from observation, but a consideration of the familiar segmental organ of Lumbricus renders

this highly probable; likewise the presence of anastomoses between the separate windings of the canal is uncertain." Lemoine (25) follows Odier in retaining the name red gland, and after a somewhat elaborate description of the shape, color in various parts, and external relations, admits ignorance regarding the internal structure. The presence of intra-cellular plexuses in the yellow mass was indicated in my paper on the Anatomy of Bdellodrilus illuminatus (28).

As the constitution of the plexus region of this species is such as to render it a more favorable object of study than the others, the account can conveniently begin with a description of it. If a nephridium, preferably the anterior one, be dissected out entire,[1] and examined while yet fresh in a drop of water, the plexus region presents the appearance of a somewhat compact and irregularly rounded, lobulated mass, from the surface of which rounded nodules stand out here and there more prominently. One is forcibly struck by the resemblance in form of such a preparation to a bulbous-rooted plant. The plexus region resembles the thickened subterranean parts, while the straightened tubule loops with their outstanding leaf-like peritoneal cells simulate the aerial stem. If now the granular mass be subjected to the pressure of a cover glass, and the nodules gently flattened, the plexus of canals will become very distinct in certain of the marginal ones, while in nearly all of them it becomes more or less clearly visible. The entire interior of the highly granular substance of the plexus nodules is found to be everywhere excavated by a system of branching and anastomosing canals. These are very tortuous in their courses, of very irregular diameter, and without any definite plan of branching. The more superficial ones form irregular arches and loops (Fig. 18), which frequently approach very close to the surface, leaving only a thin shell of protoplasm. At two points within each nodule the passages converge and unite. Most frequently these points are close together (Fig. 1, *pn'*), so that

[1] This operation can be performed much more readily upon B. illuminatus than any other species, owing to the weakness of the muscular layers. In other species the body muscles are so thick and strong that they cannot be torn asunder without serious injury resulting to the delicate organs within.

the passages are very strongly arched between them, but they may be at opposite poles (*pn''*). They correspond to the places of union between the nodules and the simple connecting tubules, one afferent, one efferent, with the lumina of which the converged passages are continuous. The plexuses increase greatly in complexity with the growth of the animals, and the nodules correspondingly gain in size and opacity. In the newly hatched individual the nodules are very slight enlargements of a tortuous canal, and the lumen very slightly branched; but as the animal grows the canal branches more and more, until the enlarging nodule is completely honeycombed by a network of passages (Fig. 18). Dorner (**14**) has already stated that the tubules are more distinctly seen in the brown mass of young individuals, owing to the fact that it becomes more massive with age, but the change is due to an alteration in the character of the tubules themselves, and not primarily to any increase in a cellular envelope, as he supposed.

Within the limits of a single nephridium the several plexuses show a certain variation in complexity, and in general it may be said that they become somewhat more simple as the external end of the series is approached, and decidedly so in the accessory plexus lobe. This is shown in Fig. 1. In the last three or four nodules composing this lobe (*ap*, *ap'*) the interior becomes more extensively excavated, but the passages can scarcely be said to constitute plexuses, consisting as they do of rounded recesses opening by wide apertures into an irregular central chamber, which communicates at two points with the connecting tubules, and in the case of the last one with the system of tubule loops (Fig. 1, *lt*).

Nuclei are absent from the plexus nodules, the one figured as present in that position in my former paper being probably an abnormal condition, or one so projected optically from a different level. The nodules are very finely and densely granular, but in this species, owing to the almost total absence of pigment, are not very opaque. This is especially true of the accessory lobe, which is quite translucent and only slightly granular. It is in every respect a transitional region between plexus and simple tubule.

The simple tubules (Fig. 1, *ct*, and Fig. 39) which alternate with and connect the plexus nodules are short lengths of "drain-pipe" cells having a diameter of .03 mm. at the middles of their lengths, and slightly more at their ends, where they enlarge to pass into the nodules. They may be straight, or more or less curved, according to local conditions (Fig. 1). The lumen by which each is perforated is a simple canal of about .013 mm. diameter, but enlarging more or less at the ends to pass into the plexuses. The walls of the tubules are more transparent than the nodules, but granular and radially striated (Figs. 16, 39).

Each tubule contains a conspicuous bunch of cilia. These are few in number, and are arranged on one side of the lumen, arising usually from its efferent end and nearly opposite to the nucleus. They seem to be arranged in a longitudinal series (Fig. 39), but in a few cases appear to be attached to a transverse ridge. Though few in number, the cilia are very long (.05 mm.), and commonly reach the entire length of the tubule; sometimes in short ones their free ends extend into the beginning of the following plexus (Fig. 1). Beating with a movement that is at once undulating and rotary, and so rapidly that I was unable to satisfactorily count the movements (about 140 per minute at 50° F.), these powerful cilia must exert a considerable force in propelling the fluids onward. Their free ends frequently become caught on the sides of the tubules, and more or less obscured in a mass of granules which collects at such points. In other cases the cilia become adherent side by side in collected mucous and granules, and act as an undulating membrane. Cilia are never found in the plexuses except in the last one or two of the accessory lobe nodules, into the excavated chambers of which they may extend as a few isolated patches of shorter cilia. The mechanical advantage and, indeed, necessity of the cilia in the narrow passages are evident, the cross section of these being many times less than the aggregated cross sections of the plexus passages, and the velocity of the stream of passing fluid as many times as great.

Each of the short tubules contains a prominent nucleus lying in the wall close to the lumen, usually near its middle, but

sometimes at one end. As no nuclei are found in the plexus nodules, it may be concluded that each of the drain-pipe cells in this region includes a short simple tubule as its middle portion, and part of a plexus nodule at each end. This, however, will be referred to again.

By careful observation of the ciliary action in the connecting tubules of removed nephridia, remembering always that the waves of movement travel toward the nephridio-pore, the tortuous course of the tubule through the plexus region may be traced. Here and there spots may be obscure, but if the nephridium has been mounted in a normal fluid,[1] it will live for a long time (one-half hour or more), and as the cover glass gradually settles down, these points become clearer. Or better yet, the tubule may be slightly unravelled by the careful use of needles, and the whole course traced. Fig. 1 was drawn from such a dissection, in which the crowded nodules were gently pulled asunder and the tubule folds opened. After drawing the living specimen, the whole was stained in methylene blue to bring out the nuclei. Such stained specimens sometimes showed the plexuses very vividly, as the stain was drawn into the passages by the ciliary action and their immediate walls stained very quickly.

Reference to Figs. 1 and 2 shows, regarding the arrangement of this region:

First: That the alternating plexus nodules and connecting tubes constitute a continuous tubule, the lumen of which is in every part distinct and enclosed in unbroken protoplasmic walls; and that there is nowhere any indication of any anastomoses between contiguous portions of the tubule folds.

Second: That in about seventeen of the nodules the plexuses are well developed, and that in this region, making up the main plexus lobe, the folds of the tubules are arranged along a portion of the efferent duct as an axis. The exact arrangement is not shown in Fig. 1, in which the parts are somewhat displaced, but the plan is indicated in the diagram, Fig. 2. Along the axial tubule (*et*^{1}, *et*^{2}) the plexus tubule bends more or less

[1] I used as most convenient at the time the blood fluid of the crayfish, diluted with an equal volume of distilled water.

regularly from side to side in a zigzag shoe-lacing fashion, the angles becoming the thickened nodules, the straight limbs the connecting simple tubules. Thus is produced an essentially two-ranked arrangement of the nodules, more or less irregular, it is true, especially as many of the nodules bend out of the common plane and conform themselves more closely to the position of their neighbors. It will be seen, however, that a more or less sinuous groove passes along one face (the mesial) of this lobe, its floor corresponding very nearly with the positions of the connecting tubules, which mostly lie side by side, and it is in this groove that the efferent duct is accommodated. The number of nodules in the main lobe is nearly constantly sixteen or seventeen. With one terminal plexus the nephrostome communicates by a short canal (*ts*); the other passes into communication with the accessory plexus lobe (*ap*).

Third: That the accessory plexus lobe consists of four or five plexus nodules with simpler internal passages, but otherwise resembling the nodules of the main lobe. The nodules of this lobe lie side by side in a single series, and the connecting tubules are usually arched. The plexus region of B. illuminatus is thus seen to have a remarkably open structure, in which it differs from all other discodrilids examined, and which is probably the more primitive condition.

In the massive region of the nephridia of B. philadelphicus, B. instabilia, and B. pulcherrima (and B. parasita seems to belong to this group), the course of the tubule is more difficult to trace. At one point is a tangled group of tubules very conspicuous in the living worm, and corresponding to the small tubule lobe of B. illuminatus. From this three tubules extend around about three-fourths of the entire margin of the compact opaque region. The latter in B. philadelphicus and B. instabilia, the only species which I have examined critically while alive, is not uniform, but, as Lemoine (25) has mentioned for B. parasita, consists of a dull yellowish portion and a more deeply colored brownish-yellow one. The former is less opaque, contains larger and simpler passages, and is closely related to the smaller tubule lobe; it consequently corresponds to the accessory plexus lobe of B. illuminatus. The latter is thicker, more granular, and so

opaque that its internal structure is made out with difficulty; but the funnel may be seen to be suspended from it, and the efferent duct to leave it. Sections of two species (Pl. XXI, Figs. 14, 15) show that the efferent duct passes along its inner face, more or less imbedded in its substance, and rather nearer to its dorsal margin; and further that numbers of simple tubules are cut along its course. This, then, is the main plexus lobe, and its interior is seen in sections (Figs. 14, 15, 17) to be excavated by systems of irregular anastomosing canals. B. parasita, as shown by the study of a series of sections, is exactly like B. pulcherrima in this respect. In all of these species the main plexus lobe is relatively small, and the accessory lobe is larger and more conspicuous; this is especially true of Branchiobdella instabilia. In Bdellodrilus philadelphicus and Branchiobdella pulcherrima the former is relatively larger and constitutes the greater part of the opaque mass. In the latter species it is relatively thicker and narrower, in the former a high flattened plate (Pl. XXI, Figs. 14, 15).

Although the separate nodules of B. illuminatus are not distinguishable in any of these species, the surface of the mass is marked off into areas by grooves of greater or less depth, which are filled by nests of connective-tissue cells, and give the body a lobulated appearance, which is frequently seen in sections to correspond with the internal arrangement of passages. Indeed, the appearance of the entire structure is such as would result if the walls of a tubule folded upon itself, as is that in the plexus region of B. illuminatus, had fused along contiguous faces, obliterating the external indications of the winding, which is then further concealed by the connective tissue and peritoneal coverings. As will be seen when the structure of the tubule loops is discussed, we there find an actual fusion of contact points along the tubules. Mutual pressure would distort the original nodules and compact and solidify the whole organ.

Whether the course of the lumen within this mass is the same as in B. illuminatus could not be determined, but the fact of alternating plexuses and ciliated canals is easily demonstrated; and it is almost certain that there are no anastomoses

between windings of what constitutes the lumen of the original tubule. The entire organ is much shortened and relatively smaller than in B. illuminatus, and the passages in all of the species mentioned relatively larger and the plexuses simpler. Cf. Figs. 13, 14, 15.

In living specimens the ciliary action in the simple lumena, so far as it can be followed, indicates a less regular folding than in the type nephridium. Nuclei and cilia have the same appearance as in B. illuminatus, but the former are larger in B. philadelphicus.

5. *The Tubule Loops.*

Starting at the last plexus nodule of the accessory lobe and extending to the intermuscular portion of the efferent duct, indeed, including this if the single enlargement be omitted, the nephridium consists of what is practically a hose-pipe tubule, more or less complexly looped and twisted, and differing somewhat in size and structure in different parts. The general arrangement of the loops has already been described, and a longer distinguished from a shorter. The two tubules of the long loop lie everywhere in close contact (except here and there where small groups of connective-tissue cells have pushed between) and extend for the entire length of both the large and small tubule lobes, passing in the former a short distance beyond the end of the shorter loop, around which they bend (Fig. 1).

As shown in Fig. 1, the paired tubules of this loop are thrown in B. illuminatus into three conspicuous folds, of which one, developed at the base of the larger lobe, is always more prominent and complex than the other two, and of these the second is larger than the third. In the small lobe also, just after the outer limb arises from the plexus, and before the inner recurves toward the shorter loop, the two become twisted about one another more complexly than is shown in the figure. It is at this point that in B. philadelphicus and B. instabilia, and, according to Lemoine, in B. parasita, the tubules become intertwined to form a prominent and intricate mass, which it is difficult to unravel satisfactorily.

In B. instabilia, owing to the colorless blood, greater transparency of the body-walls, and the thinness of their peritoneal investments, the minute characters of the tubules are more readily studied in the living condition than in B. illuminatus.

In all of the species the two tubules lie in close contact, only separating slightly here and there, where the spaces between become filled by nests of connective-tissue cells (Fig. 1, *ctn*). While in most parts the adjacent walls of the two tubules remain distinct, they frequently fuse for a short distance, forming a simple protoplasmic septum of variable thickness between the two lumina (Fig. 1). These septa are, however, never perforated to permit of communication between the lumena on either side, the only communication being the primary one at the distal end of the loop, resulting from the simple continuity of the tubule. Moreover, the lumina never encroach upon the protoplasm of the conjoined tubule, and consequently we never find here the condition of a single cell with a double lumen, but only such a condition as would result if two drain-pipe tiles were placed side by side in the plastic condition of manufacture and pressed together until the line of junction was obliterated by the union of the clay walls; a coat of cement would then represent the investments. That the integrity of each tubule is maintained throughout is further shown by the distribution of the nuclei, which is such as to enable one always to refer them decisively to one tubule or the other. In the longer tubule loop of the anterior nephridium of B. illuminatus — and the arrangement seems to be the same in other species — are found twelve nuclei (Fig. 1) distributed at fairly regular intervals (the length of which, owing to the foldings of the tubules, cannot be absolutely determined) of about .18 mm. The positions of these, as well as the windings of the tubules, are shown in Fig. 1, and are remarkably constant in a large number of individuals. Each limb of the loop may be seen to contain one nucleus of each of the six pairs in which they are disposed. They lie in the protoplasmic wall close to the lumen and on one or the other side; but whether always on morphologically the same side or regularly alternating cannot, owing to the twisting of the tubule, be confidently stated.

I have been thus particular in describing the integrity of the tubule in all its parts, because Bourne (11) and others have shown that in Clepsine, etc., the tubules in their recurrent courses pass through the same cells, and that consequently the drain-pipe cells in some regions are perforated by two passages, in which the currents run in opposite directions.

The tubules of the longer loop have a fairly constant diameter of about .02 mm., showing only slight increase or decrease here and there. The thickness of their walls is, however, more inconstant (Figs. 1, 36, 37), but is nowhere great. The protoplasm is fairly transparent and homogeneous (Fig. 36). Sections stained with haematoxylin (Figs. 22, *lt*, 37, 38) exhibit a reticular arrangement, and show a not very great affinity of the protoplasm for the stain. In living tubules of B. illuminatus the inner surface of the wall shows peculiar rod-like markings, which have proved to be a thin coating of bacilli (Fig. 54). These bodies are dense and very refringent, and their general arrangement is much like the bricks on a sidewalk — in zigzag parallel lines. They are most numerous at the ampulla-like enlargement at the point of turning of the long tubule, but may be seen in other regions also. None are apparent in any of my sections. Bourne (10, 11) has described similar markings in the living tubules of Hirudo and Pontobdella.

The lumen is throughout of somewhat irregular calibre, constricted slightly here, widened there; and it winds so that it is sometimes nearer the surface on one side, sometimes on the other. Its average diameter is .012 mm. Midway between two nuclei slight diverticula of the lumen are sometimes developed, but this peculiarity is more prominent in following regions of the tubule.

At points opposite to the nuclei (Pl. XX, Fig. 1) the lumen usually enlarges slightly to form more or less well-marked chambers (Fig. 36). These are sometimes very prominent in B. instabilia, in which the whole tubule may be expanded at these points. In B. illuminata they are frequently bounded on one or both ends by more or less prominent collars or diaphragms, sometimes developed excentrically, sometimes of equal height all around (Figs. 1, 36). On the thickest part of the

more internal one, and it is this one which is most frequently developed, a group of long lash-like cilia are attached. These are placed in a more or less sharply defined longitudinal tract, and are like those of the connecting tubules of the plexus region. A second diaphragm may be developed opposite to the point reached by the free ends of the cilia, but this is always less prominent than the other, is usually very faintly visible, and is very often wanting. Occasionally it will bear a group of smaller cilia, but this is a rare condition. Sometimes the longer cilia will project through the perforation in the second diaphragm, and their tips may be seen beating spirally on the far side. Usually they are confined within the chamber between the two diaphragms when these are present. These peculiar structures probably act as valves, though they would seem to permit the flow of fluids either way with equal facility. They may, as well as the enlargements of the lumen in which the cilia lie, be simply the mechanical result of the activity of the latter.

In the corresponding region of the post-genital nephridia the tubules have a diameter of only .0125 mm., and their lumen of .009 mm. Their structure, however, does not differ, but the arrangement is more irregular, and the two limbs of a loop are often independent for considerable distances.

The shorter tubule loop is confined to the large lobe, in which it extends from the base not quite to the end of the longer loop, and, as it is nearly straight, is considerably shorter than the latter. Its outer limb is connected with the inner limb of the long loop by a short connecting tubule (Fig. 1, *cct*), which lies in the small lobe in contact with the accessory plexus lobe, to the irregularities of which it conforms, and around the most internal nodule of which it passes before joining the short loop. In its structure this section of the tubule shows transitional characters, in that its internal end resembles the tubule of the long loop, its external that of the short loop. It possesses never more than one nucleus and one group of cilia, the position of which is shown in Fig. 1. The inner limb of the shorter loop crosses the outer at the base of the large lobe and passes into the efferent connecting duct (Fig. 1, *et*^{1}).

The tubule of the shorter loop is of smaller diameter (.016 mm.), and its wall relatively thicker than the longer. The lumen is consequently very much narrower (.006 mm.), and is more regular and generally less tortuous. The nodal diverticula mentioned above as sometimes occurring in the longer tubule are, however, more frequent and distinct (Fig. 1, *ld*^{1}); two, three, or four such diverticula frequently occur halfway between two nuclei, and may reach almost to the surface of the tubule. They differ somewhat in form, but are usually pouch-shaped, with constricted openings into the lumen. Cilia are entirely absent from the inner, and most of the outer, limb of this loop, only one group being present in B. illuminatus, near its plexal end (Fig. 1, *sc*^{1}). These cilia are similar to those already described, but, unlike those of the long loop, do not lie in a chamber, although borne upon a prominence placed nearly opposite to a nucleus. The nuclei are ten, placed at intervals of .9 mm., and at corresponding points in the two limbs (Fig. 1, *sc*^{1}, and Fig. 34). They resemble those of the longer tubule.

While the protoplasmic wall of the long tubule is only slightly granular, that of the short one is very densely and finely so, and stains very deeply. Cross sections show a dense structure with a very evident arrangement of the granules in radial lines (Figs. 22, 35). In the living state its substance is evidently more resisting than that of the long tubule. Its walls may be seen to contain minute excreted granules (Fig. 34), exactly similar to those found within the enlarged peritoneal cells. Similar granules are found in large numbers within the lumen of the short tubule, and sometimes the entire cavity of the nephridium is crowded with them.

6. *The Efferent Ducts.*

While the tubule loops are closely similar in the different species, the efferent ducts present considerable differences in size and structure. In all we can conveniently distinguish the three regions of connecting tubule, free coelomic tubule, and intermuscular tubule.

The first (Fig. 1, et^1, et^2) is very conspicuous in extracted living nephridia of B. illuminatus, in which it can easily be traced from the inner limb of the short tubule, passing first to the parietal side of the first two or three plexus nodules, then appearing on the intestinal side of the main plexus lobe, along which it passes in a groove as before described, pursuing a slightly sinuous course to the point of attachment of the funnel, where it leaves the plexus region and passes into the free coelomic section. This latter in B. illuminatus forms two folds, which closely embrace the funnel stalk (Figs. 1, 6), and in B. instabilia (Fig. 9) a more complex coil, from which only the end of the funnel projects. These folds are firmly bound together and to the funnel by their investments, so that they cannot be disengaged without injury. There is, however, no actual fusion of the walls. In B. illuminatus the tubule is sometimes thickened in this region, and cross sections present the appearance shown in Plate XXII (Fig. 23, et^1). The remainder of the coelomic tubule is a free loop which bellies ventralwards and reaches to the body-wall of the third somite (in the case of the anterior nephridium first penetrating the septum), where a slit-like opening between two of the longitudinal muscle fibres permits it to enter the intermuscular space. It is interesting to note that in B. illuminatus the tubule enters the body-wall at the level of the ventral opening of the lateral glands, and immediately anterior to those of the third somite. As the nephridio-pores of the Enchytraeidae, etc., always open just anterior to the ventral setae bundles, and are always reached by a duct which passes directly through the body-walls, this fact is further evidence that the lateral glands are homologous to the setigerous glands of the Enchytraeidae. Hubrecht (23) has shown the interesting condition in Lumbricus and Allolobophora, that while the nephridio-pores lie at any one of three levels, the efferent nephridial ducts all enter the body-wall at the same level, which is the lowermost of the three, and undoubtedly the original one, and pass thence to the upper two levels between the muscular layers of the body, just as they do in the case of the anterior nephridia of the Discodrilidae. The posterior nephridia, however, retain the primitive opening at the

level of the ventral gland pores, and the efferent duct perforates the longitudinal muscle layer directly opposite to this point.

Bdellodrilus illuminatus exhibits another peculiar feature at the point where the coelomic tubule passes into the intermuscular. Here the diameter of the tubule about doubles (Pl. XXII, Fig. 27), and its walls become very granular; the lumen develops curious and complicated enlargements, which are sometimes in the form of lateral diverticula of greater or less complexity (Fig. 28), or irregular chambers of various forms (Fig. 29), or loops and rings of divers shapes (as Fig. 30), or there may be combinations of all these.

The complexity of these structures increases with the growth of the animal, the original diverticula enlarging, and new and more complex ones appearing. In worms of about two millimeters in length there is usually only one or two small diverticula of the lumen, with perhaps a simple ring (Fig. 30[a]). In those of three millimeters the ring may have become double (8-shaped), and the lumen dilated at the position of the diverticula of the younger stage. In examples of three and one-half to four millimeters the dilatations become highly irregular and coecal, and the loops, if present, more complex; but there is no constancy in these stages, and the same individual may present marked differences on the two sides. This arrangement would doubtless be useful as a means of preventing the collapse of the tubule owing to pressure of the muscles to which it is here subjected. In the posterior nephridia similar complexities of the lumen are developed on the efferent duct where it penetrates the thicker masses of vertical muscle fibres. In B. philadelphicus (Pl. XXII, Figs. 32[a], 32[b]) the tubule is simply enlarged, and the capacity of the lumen greatly increased. This simpler condition also exists in Branchiobdella.

Throughout the remainder of the efferent duct the lumen remains simple, as in the short tubule loop, and as in that loop is provided with paired or whorled coecal diverticula at somewhat regular intervals, that is, midway between each two nuclei. The diverticula are more prominent in this than in any other section of the tubule, and an additional feature is emphasized, namely, that the lumen is more or less constricted on

each side by valve-like structures. In the connecting section of the efferent duct the tubule (in Bdellodrilus illuminatus) is smaller (of .02 mm. diameter) and the lumen larger (.009 mm.) than in the intermuscular section. In the terminal section the lumen winds more from side to side, having, as shown in Fig. 47, Pl. XXIII, a quite wavy course. Shortly before entering the pulsatile vesicle the lumen of the efferent duct forms a chamber, sometimes of twice the diameter of the rest of the lumen ; it opens by a minute pore guarded by a valve into the posterior chamber (Figs. 47, 48).

The living efferent tubule presents much the same appearance as the shorter loop, but is more transparent, and the radial striations are much more distinct. The free coelomic section and the enlargement terminating it are more highly granular, and in sections (Fig. 23) the walls are seen to be differentiated into deeply staining radial strands, forming a coarse reticulum, and a granular, less intensely stained portion, filling the meshes. Nuclei are distributed as follows in B. illuminatus : in the connecting tubule, 4 ; in the intermuscular tubule, 3 or 4 ; in the coelomic tubule, 2. In the latter, which is the shortest of the three, they are nearest together.

Although all descriptions[1] of B. parasita state that the efferent duct is ciliated throughout, in B. illuminatus cilia are entirely absent, except very rarely two small patches opposite to the nuclei of the coelomic section. B. instabilia has these always normally and strongly developed. In this species the coelomic tubule is longer and forms quite a wide loop beneath the intestine (in part Fig. 9).

In B. instabilia, B. pulcherrima, and especially in B. philadelphicus, the intermuscular tubule is exceedingly conspicuous, being, as described for B. parasita, much larger than the looped tubules. The lumen is disproportionately large, and the walls thin and not obviously striated (Pl. XXIII, Figs. 43, 44). The diverticula of the lumen are conspicuous, but short, and the lumen shows a ring-like constriction from which they arise. Before entering the terminal vesicle the lumen is greatly constricted (Fig. 43). The connecting tubule in these species is

[1] Except that of Voinov.

in great part deeply buried in the plexus mass, where it is very conspicuous in sections (Figs. 14, 15).

Considering once more the entire extent of the simple tubule, we find it made up of greatly elongated tubular (drain-pipe) cells, each with a nucleus placed at about its middle, and in many cases with a group of cilia at the same point. We cannot doubt that the diverticula of the lumen indicate the terminals of conjoined cells, and possibly they are indicative of imperfect union, though developmental facts do not support this view. Oka's recent figures of the nephridium of Clepsine tesselata show that the lateral branchings of the lumen of the main lobe are developed at points corresponding to the boundaries of conjoined cells (*Zeit. f. wiss. Zool.*, LVIII (1894), pp. 79–151). Measured by this standard the cells will be seen to be longest in the long tubule loop, where they measure about .18 mm., which is perhaps the longest simple drain-pipe cell that has been described. They are shortest in the coelomic efferent region, and the short tubule loop, where they measure about .1 mm.

The plexuses of the plexus region are to be compared with the nodal diverticula of the tubule region, which is shown by their position and mode of development ; they begin as simple diverticula which enlarge, become divided, branch, and anastomose during the later development of the worm. In both regions the nuclei and cilia are similarly placed. The comparison will be clear on consulting the diagrams (Figs. 3, 3[a]).

7. *The Terminal Vesicles.*

In all Discodrilidae, so far as known at present, the efferent tubules of the posterior nephridia terminate in separate small vesicles which communicate with the exterior by pores. The same is true of the anterior nephridia of B. parasita, B. instabilia, and B. pulcherrima. But the American genera Bdellodrilus and Pterodrilus present the unusual condition in annelids of a single median vesicle common to both. Bolsius (9) has described a median ventral opening common to a pair of posterior nephridia of Mesobdella; and Vejdovsky (34), a median

dorsal pore by which the embryonic head kidneys of Lumbricus open. In the simpler and more primitive condition exhibited by Branchiobdella and in the posterior nephridia of other genera the vesicle is of small size, less than twice the diameter of the efferent duct, and of a depressed globoid shape. The external pore is either terminal, as in the anterior pair of B. instabilia, in which case the vesicles are somewhat pointed, or near the middle of the external face. In all cases the vesicle (Figs. 40–42) consists of a simple invagination of the epidermis with its cuticular covering, forming the lining epithelium and the greater thickness of its walls. External to this is a thin connective-tissue sheath with small nuclei (*ct*) and fibrous strands running to the adjacent muscles. The vesicle is closely related to the layer of circular muscle fibres, between two of which the external pore perforates the skin; but whether a muscular coat is present on the vesicles themselves, as in the larger anterior one of Bdellodrilus, is doubtful. Sections (Fig. 40) do not indicate the existence of one, and if such be present it must be very thin indeed. A delicate sphincter surrounds the external pore, which may be circular, or wrinkled, or slit-like, or completely closed, according to the degree of contraction of this muscle. I have frequently watched the anterior vesicles of B. instabilia and the posterior of B. philadelphicus without noting the slightest contraction, although Lemoine states that the similar ones of B. parasita are contractile; but as he includes the efferent tubules (which have no muscular coat in our species) in this statement, the observation may require verification. One would, however, on *a priori* grounds expect the vesicles to be contractile. In all cases the lumen of the efferent duct is much constricted at the point where it ceases to be intracellular and becomes intercellular, *i.e.*, where the walls of the drain-pipe cells become continuous with the vesical epithelium; and here a valve-like outgrowth guards the aperture.

In B. philadelphicus the common vesicle of the anterior pair of nephridia is tubular, and hangs vertically from the dorsum of the third somite into the spacious intermuscular space (Figs. 44–46), reaching almost to the inner layer of longitudinal muscle fibres, where the two efferent ducts open close together,

in fact conjointly, into its lower end. It is lined, except at the extreme lower end, by the invaginated epidermis and cuticle (Figs. 44–46, *ve*), and is clearly surrounded by a thin muscular (*vm*) as well as by an outer connective-tissue (*ct*) coat. The position of the muscular layer, in close contact with the epithelium, indicates that it originates from the circular layer, but it is noticeable that the vesicle is pressed upon by the fibres of the outer longitudinal layer, between two of which it passes (Fig. 44, *lme*). A number of small fibres are seemingly displaced from this layer and lie more deeply, in contact with the sides of the vesicle (Fig. 44, *m*).

The anterior vesicle reaches its highest development in B. illuminatus, in which, owing to the shallowness of the intermuscular space, it is forced into a horizontal position (Fig. 47), and causes the longitudinal muscles to bulge slightly into the body cavity. Thus its greatest length is directed anteroposteriorly instead of vertically; and instead of being tubular it becomes flattened-gourd-shaped, with a somewhat narrowed neck, as seen in longitudinal section (Fig. 51). Its pore is situated on a rather distinct papilla having the same situation as in B. philadelphicus. The general structure is the same as in the latter species, but owing to the much larger size of the epidermal cells, the lining epithelium is thicker (Figs. 50, 51), and the number of cells fewer; and moreover they at one point extend into the lumen in such a way as to form an internal diaphragm, which imperfectly divides the interior into two chambers communicating through a central opening (Figs. 47, 48, 51). The anterior and larger compartment (*e*) opens to the exterior, and is alone lined by cuticle. The inner one receives the efferent tubules, which in entering the vesicle become first attached to its walls at an anterior point, where the muscular coat is perforated. They then bend sharply backward (Figs. 47, 48), the tubule walls passing into the epithelium, and the lumen extending between the cells to enter the posterior or inner chamber, one on either side (*ec*). In this species the muscular layer (Figs. 50, *vm*, 51, *m*) is well marked, and is derived mainly from the circular fibres, though the longitudinal are closely related to its ventral wall. Of all species the vesicle of

B. illuminatus is the most actively contractile, and may be seen in specimens slightly flattened under a cover glass to contract spasmodically with some regularity, and as frequently as two or three times per minute. The diastole takes place slowly, the systole suddenly and vigorously. Figs. 48 and 49 represent the appearance of the vesicle in diastole and systole respectively, as seen in dorsal views. In no case has any contraction of the efferent duct been noticed.

In both B. philadelphicus and B. illuminatus three or four small, unicellular, pear-shaped glands (*g*) are related to the anterior vesicle. In the former they open into the lower end of the vesicle at the point where the efferent ducts enter, in the latter either into the neck or just before the pore. No similar cells have been found elsewhere in relation to the nephridia.

8. *Investments of the Nephridia.*

There are two investing membranes, an internal connective-tissue sheath, which covers all parts, and a peritoneal endothelium, which, of course, is confined to the coelomic portions.

The former is similar in all species; it consists of a thin granular layer, becoming at places fibrous, and here and there incomplete for small spaces, as for example, at the ends of the tubule loops. In the species with compacted plexus masses, this sheath is rather thicker, more richly nucleated, and more uniform. Any irregularities of surface, clefts and spaces between the tubules are filled by connective tissue (Figs. 1, *ctn*, *ctl*, 13–15), and in B. illuminatus sheets extend into the loose, open plexus mass; even in Branchiobdella scattered nuclei will be noted here and there within the mass. The tubule loops are everywhere uniformly ensheathed (Figs. 20, 24, 27, *ct*), but it is interesting to note that this layer is absent from the expanded portion of the funnel, beginning only at the point where the latter meets the efferent duct (Fig. 10). A thin lamina also lies between the double peritoneal sheets which suspend the plexus mass from the body-walls. The intermuscular tubule is likewise invested in a connective-tissue coat continuous with that covering the coelomic structures

(Figs. 27, 32). Fibrous strands and sheets with nuclei scattered here and there anchor the tubule to both muscular walls, and small muscle fibres situated at intervals along its sides serve to adjust the tubule to the body movements. These are best developed in B. philadelphicus, and are shown in Fig. 44.

The parietal and septal peritoneal endothelium is a thin but dense protoplasmic layer of very uniform thickness of about .001 mm. in all of the species. Here and there, separated by considerable intervals, are marked convex thickenings containing conspicuous nuclei. A single transverse section seldom shows more than one of these, and four or five successive sections may not infrequently be searched before even a single peritoneal nucleus is evident. In sections conspicuous striae connected by interfibrillar meshes are seen passing from the neighborhood of the nucleus, and as these show equally well in longitudinal and cross sections, they probably radiate in all directions. The peritoneal cells must consequently be very large but excessively flattened, and slope upon all sides to a central nucleated thickening. Cell boundaries have not, however, been demonstrated, even with silver impregnations.

At the point where the efferent duct enters the body-wall the parietal peritoneum is reflected inward over the tubule (Figs. 27, 32) and the entire nephridium, from the plexus region of which (this refers only to the anterior nephridia) two broad but very thin double suspensory sheets pass to the body-wall, one from the funnel end, the other from the opposite end (Fig. 33). At the termination of the longer tubule lobe, the peritoneum leaves the nephridium (except in B. illuminatus) as a double fold and passes again into the parietal layer, at the base of the adjacent septum. In B. philadelphicus muscle fibres are occasionally seen in the supporting sheets of the peritoneum.

Except that the nucleated thickenings are densely filled with conspicuous granules and have more the appearance and color of chlorogogue cells, the nephridial peritoneum does not differ in B. philadelphicus from the parietal. In a full-grown specimen of this species nine or ten nuclei can be counted, of which

half are on the plexus mass. These are very conspicuous and remarkably constant in position; Fig. 15 shows one at *p*.

In B. instabilia a distinction is noticeable between the plexal and tubule peritoneum, the latter being thicker and perhaps more granular, and with an occasional outstanding cell. The nuclei are closer together, and at least twice as numerous as in the last species.

Bdellodrilus illuminatus differs again remarkably in the character of its nephridial epithelium. On the plexus region there is nothing noteworthy, and in young examples (just hatched) this is true of the tubule region also. As the worms increase in size the number of cells investing the latter region becomes greater, and at the same time they begin to grow out prominently, first at the proximal end, and finally along the whole length of the tubule lobe. The cells are at first rounded and bulging, but finally elongate and stand out prominently from the sides of the tubules. In the mature worm (Fig. 1, *p*) many of them are greatly elongated, with thickened basal ends which fuse to form a common layer covering the tubules, but remarkably thin and flat leaf-like distal ends which often branch. The largest of these cells are grouped together principally at two points, namely, at the distal end of the large lobe and at its middle (Fig. 1). Here their free ends are attached to the body-walls, and those of the terminal group are partly arranged side by side (but not coalesced) as a transverse sheet, uniting by their free ends partly with the adjacent body-wall, partly with the heart, and possibly one or two with the oesophagus. These cells anchor the nephridium in place, and at the same time present a great surface for excretion. They are easily detached, however, and the torn ends present a frayed and ragged appearance (Fig. 1). Many of the cells never acquire these connections, and may be seen washing back and forth with the currents in the coelomic fluid. Dark lines, mostly longitudinal and wavy, mark the surfaces of the flattened portions of these very pellucid cells, and are probably wrinkles, like those on a piece of crape cloth. In addition the protoplasm is filled with numerous granular excretions, which in the full-grown worm are very minute, very abundant, and gathered into little clusters (Fig. 53).

9. *General and Comparative.*

I regret that my own physiological and embryological studies of the discodrilid nephridium are not sufficiently advanced for publication at the present time. In a general way it may be stated that the results of the former agree with the now generally accepted notion of the excretory function of the nephridium, but it is desirable that the special physiological parts played by the several structurally distinct regions of the tubule be determined. This nephridium stands in a twofold relation to the excretory process. The first is a direct one, in that the nephridial tubules eliminate, and finally deposit within their lumina in the form of granules, waste matters contained in solution in the coelomic fluid, which bathes them without and passes in a constant osmotic stream through their walls. This is indicated by the conspicuous radial striation and by the presence of deposited granules within the protoplasm. This has been shown by Benham (6) for Lumbricus, and by several other observers in allied genera. Of probably still greater importance quantitatively is the passage of waste matters in solution through the tubule walls into their lumina without deposition. This is perhaps surmised rather than proved by the fact that alcohol causes the appearance in the protoplasm of abundant granules, before invisible, indicating their precipitation from a state of solution.

The second is an indirect relation, in that the nephridia serve simply to conduct to the exterior of the body the products of excretion elsewhere accomplished. The chlorogogue cells stand, as has been frequently pointed out, in a most intimate relation to the perienteric blood sinus, and also, by means of protoplasmic strands, to the absorptive surface of the alimentary canal. The existence of strong diffusion currents through them from the alimentary epithelium, and especially from the blood sinus, to the coelom, is indicated by the arrangement of the granules. These form lines extending from the haemal to the coelomic surfaces of the cells, many of them curving around the nuclei, as though these obstructed free movement in straight lines. The cells are minutely and abundantly vesicular, and

filled with granules which impart to them a green-brown color. The coelomic corpuscles have exactly similar characters, and one finds frequent indications of their continuous development by budding from the chlorogogen cells, just as Schaeppi (31) has shown them to arise from other regions of the peritoneum in the Polychaeta. By this means the chlorogogen cells give up a part of their load of waste substances to the freely circulating coelomic corpuscles, many of which are seen in various stages of disintegration, thus finally freeing, partly in the solid state, partly in solution in the coelomic fluid, the waste matters which they hold. These products of disintegration are continuously drawn into the nephridial current, passing into the nephrostome, and from time to time expelled from the external pore. Accumulations of disintegrated coelomic corpuscles are abundantly found in all parts of the nephridial lumen, and clouds of minute granules are often seen to be ejected from the nephridio-pores.

As the corpuscles are produced and broken down not only in the nephridial somites, but also in the male, and to a less extent in the female somites, which contain no nephridia, and as the septa limiting these somites are nearly imperforate, the suggestion arises that the genital ducts may to some extent convey the coelomic fluid with its contained waste matter to the exterior, and be in that sense excretory. The ciliary action within the vasa differentia and the peristalsis of their walls must necessarily force some of the fluid along with spermatozoa to the sperm reservoir, and thence to the exterior; and, as the ciliary action is constant and the duct of considerable capacity, this stream may reach a not unimportant volume. With each extruded ovum also a small quantity of fluid must be forced through the ovipores, and if these remain open for any length of time a considerable but intermittent current would be induced by the pressure of the body-walls. It must be recalled, however, that chlorogogen activity is reduced to a minimum in the ovarian somite.

The following facts are significant in this connection. Among the lower Oligochaeta the nephridia very generally lack blood supply. Coelomic excretion is therefore predominant, and the

chlorogogen layer extends far forwards. In such worms nephridia are absent from the genital somites, and the genital ducts have widely open mouths suitable for conducting away the coelomic fluid. The funnels are sometimes glandular (Enchytraeidae). In the higher, mostly terrestrial Oligochaeta, nephridia are frequently present in the genital somites, but the male ducts are frequently cut off from communication with the general coelom by being enclosed in spermatic vesicles, periodically at least. The nephridial funnels are more highly specialized for producing currents in the coelomic fluid, but the absence of chlorogogen cells in this region leads one to believe that the fluid may here be simply watery, without excreta, and serve in the nephridium the purpose of flushing the tubules, as does the fluid secreted by the Malpighian vesicle in the vertebrate kidney tubule. An elaborate blood supply transfers the source of waste substances supplied to the nephridium largely from the coelomic fluid to the blood. Posterior to this region the conditions are changed, for here the chlorogogen cells are abundantly developed, but the dorsal pores, more constantly developed in this region than anteriorly, offer a means of exit to the surplus fluid and excreta which they pass into the coelom. Beddard (1) has already suggested the probable excretory function of the dorsal pores, but the other function, that of assisting to moisten the surface of the body, which has been assigned to them, is doubtless equally important, as this has been shown by Whitman (35) and others to be the final disposition of the fluid accumulated by the nephridia of certain leeches.

The condition of the Hirudinea is especially interesting. Here we find in very close relation to the decreasing importance of the coelom, and of the coelomic fluid as a means to excretion, a frequent occlusion of the nephrostomata, a wonderfully rich nephridial blood supply, and a closure of the coelomic ends of the vasa deferentia, owing to continuity with the testes. Here, of course, the testes and nephridia are developed metamerically side by side. The almost exclusively coelomic excretion of the Polychaeta, associated with the utilization of the nephridia as genital ducts, needs also to be mentioned.

The discodrilid nephridium has probably no other than an excretory function, there being no evidence that it may be respiratory, though the fluid which it carries off is probably more or less laden with carbon dioxide. The statement by McIntosh (26) that in Branchiobdella the posterior nephridia serve as oviducts is probably without foundation, as no more unsuitable conductor for the large ova could be imagined than the narrow tortuous tubes ; besides they have no communication with the ovarian somite, and the true ovipores are easily demonstrated.

It now remains to compare the discodrilid nephridium with its homologue in other annelids. It is unnecessary to consider the Polychaeta, with their short wide tubes. On comparing typical nephridia of Hirudinea and Oligochaeta, the resemblance between the two is remarkable ; and this is true whether we compare the plectonephridia of the fish leeches Pontobdella, Piscicola, and Branchellion with those of such Oligochaeta as many of the Acanthodrilidae, Perichaetidae, and Cryptodrilidae, or the meganephric condition of the leeches possessing more specialized coeloma with that of Lumbricus, for example. The second type alone concerns us here, and we may select for comparison Bourne's (11) figure of Clepsine and Benham's (6) for Lumbricus. Omitting details we find that in the body of the nephridium the entire tubule system of Clepsine corresponds to the middle loop alone of Lumbricus, the main lobe of the former being the anterior, the apical lobe the posterior, limb of that loop. At the apex of the apical lobe of Clepsine both the wide and narrow tubules bend back, the latter returning on itself, the former passing from the main lobe into and down the apical lobe. Now if this apex be imagined as greatly produced, we shall have four tubules lying side by side and corresponding exactly to the third or posterior loop of Lumbricus, which is nothing but the greatly developed apex of the apical lobe. The connecting tubule or bridge between the apex of the apical lobe and the main lobe of Clepsine is represented in Lumbricus by the point where the narrow and middle tubules meet. The perforated cells of the main lobe of Clepsine are evidently enlarged drain-pipe cells which have secondarily enclosed the

efferent and recurrent tubules, and in which the lumen has developed side branches. The corresponding tubule in this region of Lumbricus also shows, according to Benham, slight indications of branching. In both types the efferent duct leaves the body of the nephridium at the same point at which the nephrostomal tubule enters. The former in Clepsine is direct, in Lumbricus looped and very wide, but this region is highly variable in both groups which these two genera represent. The funnel of Clepsine is primitive, but open and functional; while the stalk, the testes lobe of Bourne, is highly important, and differs from the main lobe only in that it is free from the other tubules, and its cells smaller. Lumbricus has a highly specialized funnel, while the stalk is a simple conducting tubule.

The hirudinean meganephridium which departs most widely from that of Clepsine is that of Hirudo and its allies on the one hand, and Nephelis on the other ; in the former the funnel has degenerated from a highly specialized condition, and its cavity is occluded, which Bourne (11) notes is connected with an increased richness of blood supply. The perforated cells of the testes, main, and apical lobes no longer form a simple series, but have increased to a number of rows, the lumen becoming similarly divided to form a network, which is most complex in the main lobe, where the recurrent and efferent tubules have each acquired a special cellular sheath. The coecal lobe is also a special addition to the nephridium of Hirudo. But the tubule arrangement is exactly the same as in Clepsine, which serves well as a type. The nephridia of Nephelis, as recently described by Graf (*Jen. Zeit.*, XXVIII, pp. 163–195) presents a very simple arrangement in that the tubule, although sinuous and folded in its course, nowhere becomes complicated by recurrent or encircling loops. Although the lumen develops lateral branches for a great part of the length of the tubule, the plexus region is confined to a small nodule which is enveloped in a network of blood vessels. The funnel is functional. In many respects this nephridium closely resembles that of certain Tubificidae as described below, but most of these are thrown into U-shaped loops.

Turning now to the Oligochaeta, we meet with a much greater range of variation, but, omitting a few special cases, the great majority of nephridia which have been described readily fall into line. In reviewing the several families of earthworms we may select a few typical genera for comparison, using those in which the arrangement of the tubules has been sufficiently described, though the finer structure is in most cases still unknown. Among the Lumbricidae there is little departure from the Lumbricus type except in Allurus (Beddard, 2), which has simplified nephridia. Many genera of the Geoscolicidae have two series of nephridia which differ somewhat; the anterior ones are frequently specialized, but the general arrangement of the tubule is in two parallel loops, as in Rhinodrilus (Thamodrilus) (Beddard, 3), and Criodrilus, (Collin, 13), or in a tuft of loops, as in Pontoscolex (Urochaeta), (Perrier, 30). If the remarkable integumental network of Libyodrilus (Beddard, 4) and related genera be disregarded, the paired nephridia of the Eudrilidae are also of the two-looped type. (For Eudrilus, see Horst, 22.) The Acanthodrilidae include worms with the nephridia either paired or diffuse, the meganephric forms having simple looped tubules, as in Acanthodrilus (Beddard, 5), Diplocardia (Garman, 20), and Kerria (Eisen, 15). The latter is of small size and the nephridial blood supply is wanting, which may possibly also be the case in the large Diplocardia. The Cryptodrilidae are much like the last family in nephridial characters. Eisen has very carefully figured and described several of the meganephric genera, — Pontodrilus (16), Deltania (17), and Argilophilus (17) having nephridia which in complexity of tubule arrangement and blood supply rival or surpass Lumbricus; and Ocnerodrilus (18), which, while of no smaller size than some of the above, has a single bent tubule loop and no blood supply.

Although the genera enumerated above are but a drop in the bucket in these days of rapidly multiplying oligochaetous genera, and are selected as a few typical examples, the writer has examined a very large part of the published figures and descriptions in coming to the following conclusions. Among many of the higher worms the Lumbricus type is departed from

but little; the chief points of variability, other than certain special developments, are in the presence or absence or relative development of the muscular efferent duct, the degree of complexity of the funnel, the relative prominence of the middle and third loops, — both of which are always present, and the latter always to be identified as a group of four parallel and usually conspicuous tubules, — the extent to which the connecting tubule is associated with the proximal limb of the middle loop, the arrangement of the cilia, the presence or absence of an ampulla on the distal bend of the wide tubule loop, and the extent and character of the branching of the lumen.

The latter is of special interest, as it has been only a few years since such plexuses were regarded as characteristic of the leech nephridium. Since Bourne's discovery in Hirudo (10), branchings of the lumen have been described for a great many Oligochaeta, and this too in many of the simplest nephridia. The plexus is always developed at about the same region, but it is interesting to note the great variability in extent and in the manner of branching of the lumen. In some Naidae (Vejdovsky, 33) and Tubificidae (Stolc, 32) the branching occurs within the confines of a single cell, in others within a number of such cells massed together by the folding of the tubule; from this all degrees of development of the plexus are found, up to the remarkable conditions presented in such forms as Pontodrilus (Eisen, 16), in which the tubules themselves as well as the lumina branch, and the very curious and instructive arrangement presented by Argilophilus (Eisen, 17), in which the continuous but very irregular lumen gives off in part of its course numerous ring-like diverticula, which completely encircle the accompanying recurrent and efferent tubules. The plexus which Bolsius (8) has described in the enchytraeid nephridium is perhaps of a different character, and has been formed by secondary anastomoses of contiguous parts of the winding lumen, rather than by longitudinal division of the drain-pipe cells with the resulting reticulation of their lumina, as in Hirudo, Pontodrilus, etc. Now the chief point to be noted is that whatever the degree of variation in other respects, the two

apical tubule loops always remain more or less prominent in relation to the continuance of coelomic excretion; while the middle lobe, which alone is represented in the leeches, and which is chiefly characterized by the fact that the efferent tubule or its representative forms therein a complete circuit instead of a simple loop, seems to develop in close relation to the increasing nephridial blood supply. This is emphasized by a study of the nephridia of the lower or water worms, in which the blood supply is entirely wanting, or occasionally, perhaps (Rhynchelmis) (34), very slightly developed. In these the middle lobe is lacking, and the nephridium consists of the simple tubule loops, generally as in Lumbricus, two in number and variously arranged. In many Tubificidae, Naidae, Lumbriculidae, and Oelosomidae a single loop is developed; this may reach a great length and be more or less folded on itself.

The curious tufted nephridia of Pontoscolex (Urochaeta) (30) and other forms are of a different type; but almost the entire structure retains the characteristic narrow loops, which are, however, much increased in number by repeated folding of the tubule, and are arranged in close tufts.

Using the presence of these simple excurrent and recurrent loops as a test (and in the absence of details of more than a few oligochaete nephridia this seems, in addition to the greater functional activity of the funnel, to be the only one), we find on comparing the discodrilid nephridium that it arranges itself at once by the side of the oligochaete type, for the two apical loops are very important and striking parts of the organ. The most peculiar feature of the discodrilid nephridium is the large size and complete integrity of the drain-pipe cells; they never exhibit any indication of branching or splitting, but are always essentially tubular cells perforated from end to end by a lumen which never (certainly in B. illuminatus) forms lateral connections with the lumina of neighboring cells, even in the plexus region. This, and especially the prominence of the apical loops, places the discodrilid nearest to the tubificid type, as exhibited, for example, by Iliodrilus (Stolc, 32) or by Camptodrilus igneus (Eisen, 19), but differing from these in the much greater development of the plexus region, which further becomes doubled

on itself to form two lobes — a step toward the formation of the middle loop of Lumbricus.

It may be instructive to compare the nephridium of Bdellodrilus part for part with Benham's figure of Lumbricus and Bourne's of Clepsine, premising that the distinction between ante- and post-septal regions as usually understood is not here of importance in the fully developed nephridium. As compared with the former, the funnel is very simple and near to the primitive type represented in Benham's partly hypothetical series. Benham does not show the origin of the central cell, but the embryological researches of Vejdovsky on Rhynchelmis and Allollobophora (34), of Bergh on Criodrilus (7), Wilson on Lumbricus (38), and of others show the origin of the funnel from a single large cell, which may be vacuolated or not, but finally splits into the marginal cells and a single tubular basal cell which passes through the septum and joins the funnel to the body of the nephridium. This probably confirms the identification of the third cell in the funnel of Bdellodrilus with the "central" cell of Lumbricus. With the excentric growth, already shown in the discodrilids, and rapid division of the marginal cells, the ring cell would naturally assume the characteristic crescentic shape, just as the first drain-pipe cell is split into two gutter cells in accommodating itself to the same growth stress. The testes lobe of Clepsine is represented only by the funnel stalk, which resembles that of Lumbricus. The main and accessory plexus lobes of Bdellodrilus represent respectively the main and apical lobes of Clepsine and the two limbs of the middle or circuit loop of Lumbricus, the lumen complications of all three being on the corresponding (the nephrostomal) tubule. This difference, however, is important, that in Lumbricus and Clepsine these regions contain three tubules, in Bdellodrilus only two, which results from the fact that the connecting tubule of the latter is associated only with the accessory and the efferent tubule only with the main plexus lobe. The relation of the latter is of interest, as the efferent duct simply passes in an open groove along the side of the plexus mass, covered by a thin connective-tissue layer and peritoneal sheath. The connective tissue forms in Lumbricus

a mass of enlarged cells binding the tubules together, while in Clepsine the protoplasm of the nephrostomal tubule has completely included the accompanying recurrent and efferent tubules. It will be recalled that in several species of discodrilids the efferent duct is completely buried in the plexus mass. In Argilophilus the appearance of the ringed passages would seem to indicate an actual growth around and inclusion of the accompanying tubules by the nephrostomal tubule; but here the cells have undergone division, though not so regularly as in Hirudo.

The apical tubule loops of Bdellodrilus appear at first sight to resemble those of Lumbricus in arrangement, there being a narrow tubule loop and a longer wide one with a slightly marked apical ampulla. The succession of these two is, however, reversed, the wide tubule leading to the efferent duct in Lumbricus and the narrow in Bdellodrilus. I cannot believe that this will become explicable on any other than physiological grounds. The efferent tubules correspond in a general way in all three, but Clepsine lacks the terminal vesicle, while the long muscular duct of Lumbricus is very much reduced in Bdellodrilus, and the median dorsal pore of the latter is quite unique, though the head kidneys of Lumbricus have a similarly placed pore (34).

The arrangement of the tubules of the discodrilid can be derived from that of Lumbricus — if we omit the reversal in position of wide and narrow tubules — in several ways, the most obvious of which is by a great shortening of the recurrent limb (apical lobe) of the latter, with the consequent withdrawal of the connecting (recurrent) tubule from the plexus limb (main lobe). This would result in carrying the basal end of the third loop from the nephrostomal end to the distal end of the middle loop, as in Bdellodrilus, while a close folding of the plexus limb would result in the compact condition seen in all Discodrilidae. The evolution has of course been in the opposite direction, the blood supply to the nephridium, upon which the change partly depends, being a later development. The mechanical effects on the arrangement of the tubules, as a result of the manner of attachment of the blood vessels, in restraining the free

spreading of the growing tubules, are indicated in Benham's figure of the nephridial blood supply of Lumbricus, but I have been unable to follow the suggestion up comparatively.

We conclude as one result of the foregoing study that the nephridial characters, as is true of so many other structures, point to an oligochaetous alliance of the Discodrilidae; while at the same time the importance of predominating chlorogogen on the one hand or direct nephridial excretion on the other, as a factor influencing the arrangement of the tubules, is indicated.

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EXPLANATION OF PLATE XX.

FIG. 1. An entire anterior nephridium of B. illuminatus, dissected, and the plexus region slightly teased apart. Drawn from a living specimen and details added after staining with methylene blue and comparison with many similar preparations. Represented in greater part as an optical section. × 195.

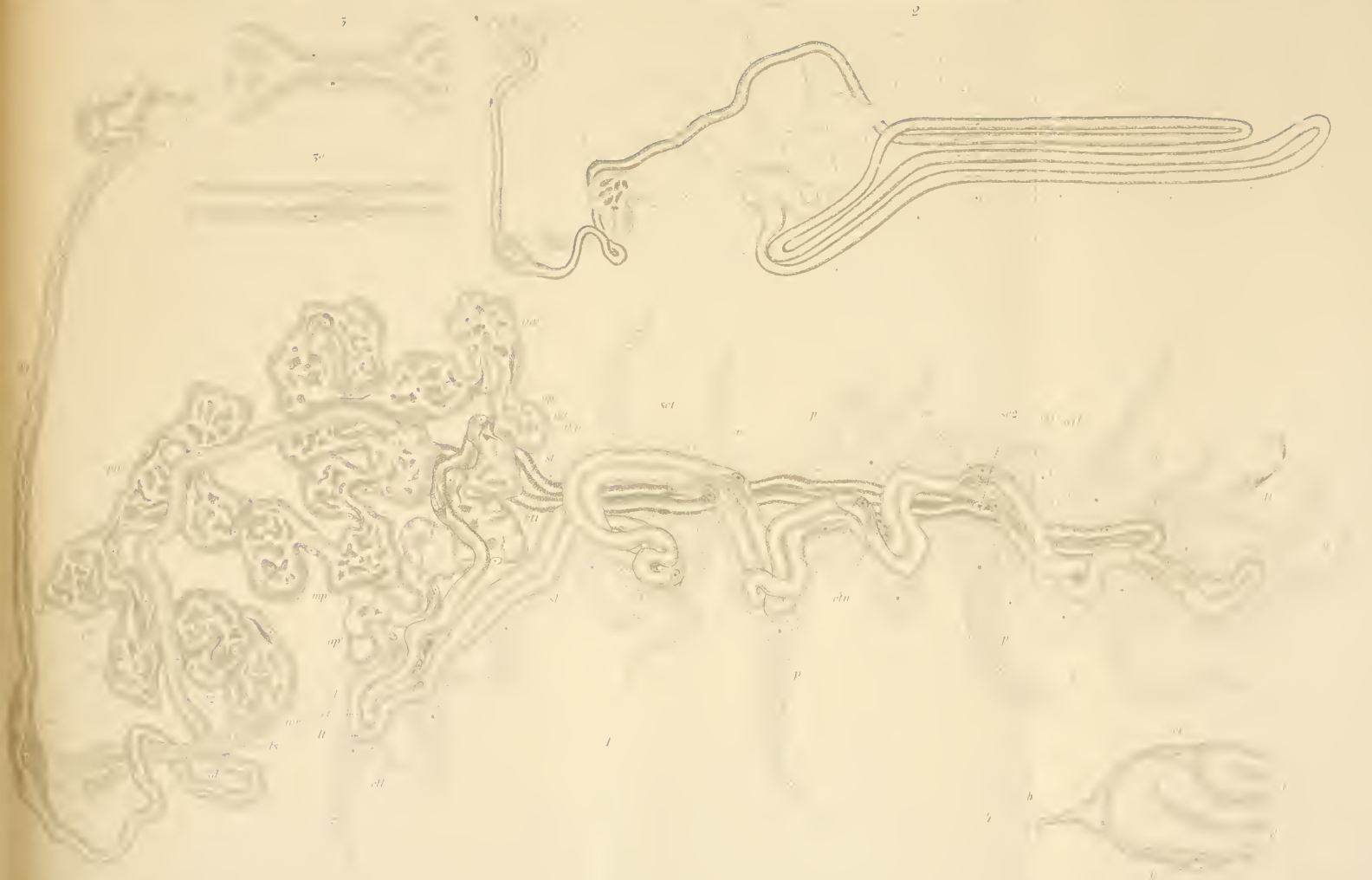
ap, *ap'*,	accessory plexus lobes.
cc,	ciliated chambers of longer tubule loop.
cct,	tubule connecting longer and shorter tubule loops.
ct,	connecting tubules of plexus region.
ctl,	connective-tissue investment.
ctn,	nests of connective tissue with nuclei.
e,	external compartment of nephridial vesicle.
ep,	epidermis and cuticle.
et,	efferent tubule; 1–2, connecting section; 2–3, folded coelomic section; 3–3, intermuscular section.
etc,	enlargement of efferent tubule at point of entry into the intermuscular space.
f,	funnel.
fs,	funnel stalk.
i,	internal compartment of nephridial vesicle.
ld,	diverticula of the lumen of the efferent tubule.
ld^{1},	ditto of the short tubule loop.
ll, *ll*,	large tubule lobes.
lt, *lt*, *lt*,	long tubule loop.
mp, *mp'*, *mp''*,	main plexus lobe.
n,	nucleus in long tubule loop.
nn,	nephrostome.
np,	external nephridial pore.
p,	enlarged peritoneal cells investing tubule loops.
pn', *pn''*,	plexus nodules.
sc,	ciliated points in short tubule loop, 1 is always and 2 sometimes ciliated.
sl, *sl*,	small tubule lobe.
st, *st*,	short tubule loop.

FIG. 2. Diagram of the same nephridium. The arrows show the direction of the currents.

FIG. 3. Diagram of a cell element (modified drain-pipe cell) of the plexus region of B. illuminatus.

FIG. 3^{a}. Diagram of a drain-pipe cell element of the tubular region of the same.

FIG. 4. B. instabilia. A small portion of the apex of the large tubule lobe, showing the ampulla-like enlargements of the long tubule as it bends around the short loop; also the peritoneal sheet connected with the body-wall. *st*, short tubule, *lt*, long tubule, *h*, peritoneum, *b*, body-wall.


3
2
sct
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b

EXPLANATION OF PLATE XXI.

FIGS. 5, 6, 7. Living nephridial funnels of B. illuminatus; 5, optical section as seen from the dorsal side (cilia of the central cell omitted); 2, profile view, optical section, dorsum toward the left, the relation of the efferent duct is indicated; 3, view of nephrostomal end. All × 500.

mn, nuclei of marginal cells.
cn, nucleus of central cell.
tn, nucleus of funnel stalk.
n, nephrostome, the arrows by the side of Fig. 7 indicate the direction of the ciliary action.
mn, uneven ciliated surface of the marginal cells.
cc, cilia of central cell.
tc, cilia of stalk cell.
et, in Fig. 6, outline of efferent tubule, in Fig. 5, its position.

FIG. 8. B. philadelphicus, funnel stained with methylene blue. The nucleus of the central cell is seen through the lumen. × 500. Lettering as above.

FIG. 9. B. instabilia, funnel showing relation to the efferent duct, which is looped around it. The cilia had just ceased to beat. × 500. Lettering as above.

FIG. 10. B. philadelphicus. A longitudinal section of a funnel, reconstructed from two successive sections. Showing the continuity of the marginal cells with the peritoneum, and the inclusion by the latter of the efferent tubule. Delafield's haematoxylin. × 880.

p, peritoneum.
pm, place where peritoneum passes into the marginal cells.
ct, nests of connective-tissue nuclei.

Other letters as in the foregoing figures. *a–a* and *b–b* respectively indicate the planes of the sections represented in 10[a] and 10[b].

FIGS. 10[a], 10[b]. B. philadelphicus. Two transverse sections of the funnel at the levels *a–a* and *b–b* of Fig. 10; *a* is through the marginal cells, *b* through the central cell. The connective-tissue investment of this funnel (*ct* 10[b]) extended nearer to the nephrostome than in Fig. 10. The cilia are of course represented diagrammatically, as in the actual sections they appeared as a close bunch of oblique sections.. × 500.

FIG. 11. B. instabilia. Horizontal section near the body-floor of postcephalic somites II, III, and IV to show the normal position of the nephridial funnels. The somites and septa are numbered. × 112.

a, alimentary canal.
et, efferent tubules at points of entrance into the body-walls.
t', *t''*, funnels.
im, intermuscular spaces of somite III.
lm, longitudinal muscle fibres.
tl, tubule loops.
vm, dorso-ventral muscle fibres.

FIG. 12. B. instabilia. A similar section through part of the somites VII and VIII. The section is somewhat oblique, owing to the natural curvature of the body. × 112.

VII, minor annulus of somite VII.
l, lateral flange of somite VIII, made up chiefly of muscle fibres.
s, longitudinal septum separating a lateral portion of the coelom in which the funnel lies; remaining letters as in Fig. 11.

FIG. 13. B. illuminatus. Part of a transverse section of the 3d somite, showing the position and character of the plexus region of the nephridium. × 112.

cg, chlorogogue cells.
ep, epidermis.
et, sections of efferent tubule.
tp, first plexus nodule into which the funnel passes.
lm, longitudinal muscle layer.
lt, long tubule loop.
p, peritoneal investing cells.
pn, plexus nodules.

FIG. 14. B. pulcherrima. Section similar to that represented in Fig. 13. × 112.

t, funnel.
pn, compacted plexus region.

Other letters as in Fig. 13.

FIG. 15. B. philadelphicus. Similar section. × 112.

ts, funnel stalk.
st, short tubule loop.

Other lettering as in Fig. 13.

FIG. 16. B. illuminatus. A section through a plexus nodule and accompanying tubule. × 500.

FIG. 17. B. pulcherrima. A section through a portion of the plexus mass. A tubule (*t*) is shown partly embedded in its substance. × 500.

FIG. 18. B. illuminatus. A living plexus nodule under slight pressure, showing the plexus formed by the lumen. × 500.

FIG. 19. B. illuminatus. A small portion of the wall of a plexus nodule, showing its vesicular structure; living and stained with methylene blue.

EXPLANATION OF PLATE XXII.

FIG. 20. B. illuminatus. Oblique section through a long loop tubule, and its investments. × 500.

ct, connective-tissue investment, in some parts not distinguishable from the peritoneum.
i, slight intra-lumenal extensions of the walls.
n, nucleus of tubule.
p, peritoneal cell.

FIG. 21. Same as Fig. 20, but a much thinner section; the lumen contains granules. × 500.

pc, peritoneal (coelomic) corpuscle.

FIG. 22. B. illuminatus. Transverse section across the large tubule lobe of the anterior nephridium, showing the different structure of the long and short loop tubules, and the peritoneal investment. × 500. This figure is taken from the Journal of Morphology, vol. x, no. 2.

lt, long loop.
st, short loop.

FIG. 23. B. illuminatus. Transverse section of the anterior nephridium at the region of the funnel stalk. × 500.

ct, connective-tissue nuclei.
ts, funnel stalk.
et, efferent tubule.
et_1, thickening of the efferent tubule at its point of turning.
pn, beginning of a plexus nodule into which the funnel stalk passes.

FIGS. 24, 25, 26. B. philadelphicus. Sections of the tubules of the anterior nephridia; 24 and 25 of the long loop, 26 of the short. × 500.

Lettering as in Fig. 20.

FIG. 27. B. illuminatus. Transverse section of a portion of the body-wall of the 3d somite at the point of entrance into the intermuscular space of the efferent duct. × 500.

cm, layer of circular muscle fibres.
ct, connective-tissue sheath.
ep, epidermis.
ets, section of the tubule.
lm, longitudinal muscles.
lp, plexus of the lumen.
p, peritoneum.
r, radial marking around the lumen.

FIGS. 28, 29, 30. B. illuminatus. Outlines showing some of the peculiar arrangements of the lumen within the enlargement of the efferent tubule.

FIG. 30 is the form most frequently assumed. The arrows indicate the direction of the current.

FIG. 30a represents the same region of a young worm (2 mm. long) but is somewhat more enlarged.

FIG. 31. B. illuminatus. The section of a post-genital nephridium corresponding to the above and represented under a similar magnification.

FIGS. 32a, 32b. B. philadelphicus. Two successive sections of the efferent tubule at point of entering the body-wall. × 500.

Lettering as in Fig. 27.

FIG. 33. B. philadelphicus. Portion of a horizontal section of somite III showing the position of the efferent tubule in the spacious intermuscular space. × 112.

cg, chlorogogue cells.
et, efferent tubule.
t, funnel.
lme, external layer of longitudinal muscles.
lmi, internal " " " "
p, peritoneal supports of nephridium.

FIG. 34. B. illuminatus. Portion of a living tubule of the short loop, showing nucleus, cilia, and granules contained in the protoplasm. × 880.

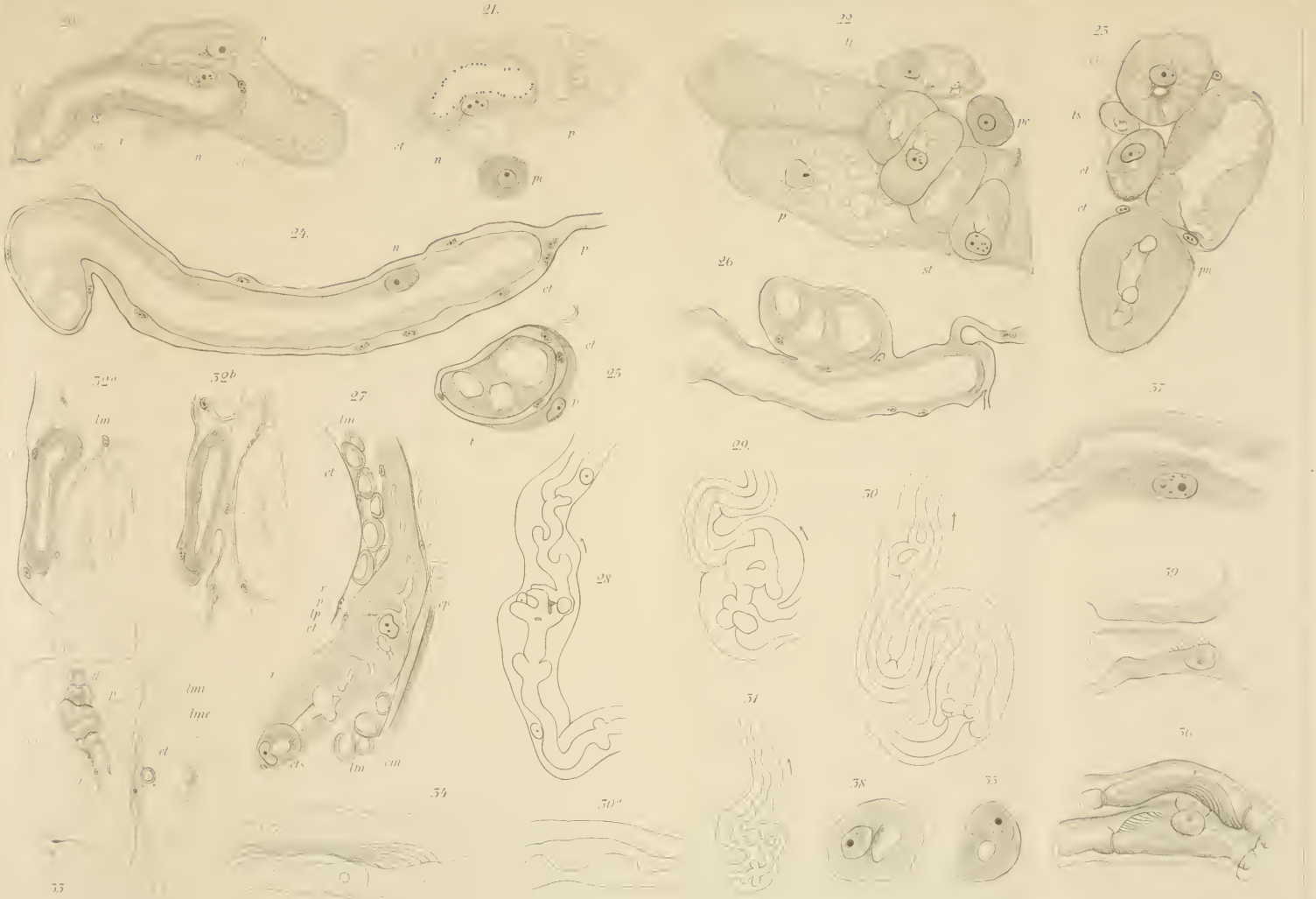
FIG. 35. B. illuminatus. Section across a tubule of the short loop. × 900.

FIG. 36. B. illuminatus. Portion of the two living tubules of the long loop, showing the ciliated chambers side by side. × 500.

FIG. 37. B. illuminatus. Longitudinal section of a ciliated chamber of the long tubule loop. × 900.

FIG. 38. B. illuminatus. Transverse section of a ciliated chamber of the long tubule loop. × 900.

FIG. 39. B. illuminatus. Optical section of one of the connecting tubules of the plexus region. × 500.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EXPLANATION OF PLATE XXIII.

FIG. 40. B. instabilia. Transverse section of one of the anterior nephridial vesicles. × 500.

FIG. 41. B. illuminatus. Semidiagrammatic figure to show the relation of a post-genital nephridio-pore to the lateral gland. Reconstructed from two vertical sections.

np, nephridio-pore.
g, opening of lateral gland.

FIG. 42. B. illuminatus. Profile (*a*) and face (*b*) views of the vesicles of a posterior nephridium, living. A cluster of excreted granules is shown at *g*. × about 275. Taken from Journal of Morphology, vol. x, no. 2.

FIG. 43. B. philadelphicus. Dorsal view of the anterior unpaired vesicle *in situ*, showing a portion of the efferent duct of one side. × 500.

ec, pore by which the lumen of the efferent duct communicates with the cavity of the vesicle.
en, nucleus of efferent duct.
ed, diverticula of lumen of duct.
g, gland cells.
vl, cavity of vesicle.
np, nephridio-pore.

FIG. 44. B. philadelphicus. A vertical section through the vesicle in a transverse plane of the body, showing the relation of the vesicle to the intermuscular space and the efferent ducts of the two nephridia. × 500.

c, cuticle.
cm, circular muscle fibre.
ct, connective tissue, nuclei, and sheath.
ep, epidermis.
et, efferent tubules meeting the vesicle at *ec*.
g, glandular cells.
lme, external longitudinal muscle fibres.
lmi, internal " " "
m, isolated muscle fibres related to ducts.
np, nephridio-pore.
p, parietal peritoneum.
ve, epithelial lining of vesicle.
vm, muscular sheath of vesicle.

FIG. 45. The same. A transverse section (in a horizontal plane of the body). × 500.

eg, portion of a skin gland.

Other lettering as in Fig. 44.

FIG. 46. The same in vertical longitudinal section of the body. × 500.

Lettering as in Fig. 44.

FIGS. 47, 48, 49. B. illuminatus. Outlines of the living vesicle from the side and from above. 48 in diastole, 49 outline in systole. × 500.

e, external, and *i*, internal, compartments.

Other lettering as in Fig. 43.

FIG. 50. B. illuminatus. Transverse section through the anterior chamber of the vesicle. × 500.

Lettering as in Fig. 45.

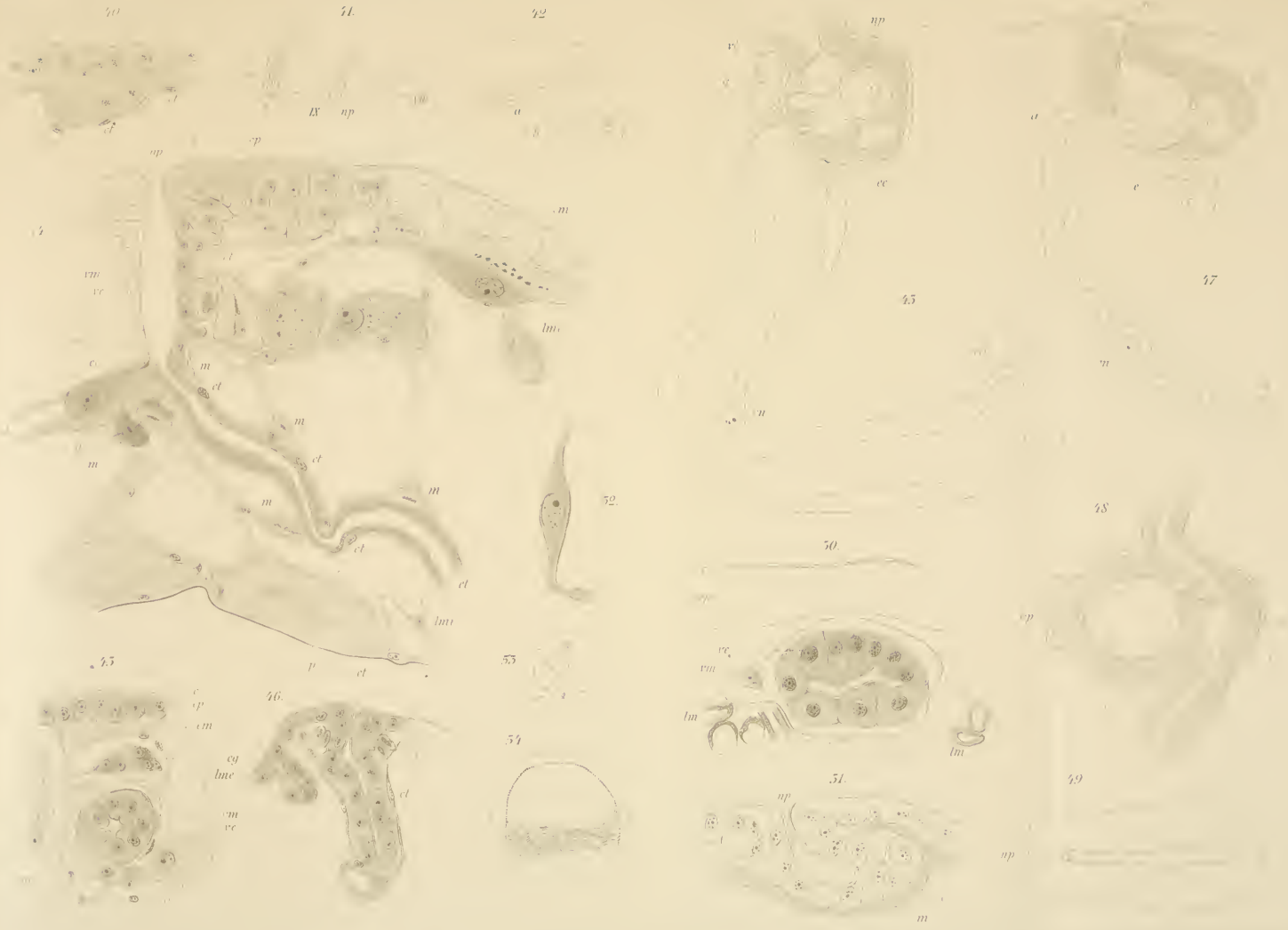
FIG. 51. B. illuminatus. Longitudinal section of the anterior nephridial vesicle. × 500. From Journal of Morphology, vol. x, no. 2.

c, internal compartment.
m, muscular layer.

FIG. 52. B. philadelphicus. A section through the nucleated portion of peritoneal cell; from the nephridium. × 880.

FIG. 53. B. illuminatus. A few clusters of granules from the enlarged peritoneal cells of the nephridium. × 900.

FIG. 54. B. illuminatus. A small portion of the apex of the long tubule loop, showing the bacilli-like markings of the inner surface, represented partly as an optical section, partly as a surface view. × 900.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STUDIES ON THE ELEMENTS OF THE CENTRAL NERVOUS SYSTEM OF THE HETERONEMERTINI.

THOS. H. MONTGOMERY, Jr., Ph.D.

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I. Introduction.

The present paper gives the results of investigations upon the finer structure of the elements of the central nervous system of *Cerebratulus lacteus* (Verrill) and *Lineus gesserensis* (O. F. Müll). It is more especially the structure of the ganglion cells and of the nerve tubules which has received attention; but

the distribution of the neuroglia, and of the ganglion cells II and IV, as well as the one or two posterior commissures of the ventral brain lobes, hitherto undescribed, have also been studied to a certain extent.

To the central nervous system of the nemerteans must be reckoned: (1) the dorsal and ventral brain lobes and their commissures; (2) the lateral nerve chords which, in both species examined, here unite posteriorly just beneath the end of the posterior intestine; (3) the paired oesophageal nerves; (4) the longitudinal nerves of the proboscis (two in number in each of our species); (5) the dorsal, unpaired, larger, median nerve of the body wall; and probably also (6) the lesser, unpaired, median nerve. Thus in the nemerteans the central nervous system, *i.e.*, that portion of the whole nervous system with which ganglion cells are in contact, has an extent and diversification which closely approach that of the Mollusca. Previous authors have limited the term "central nervous system" to the brain lobes and lateral chords, disregarding the fact that the other parts, mentioned above, are also provided with ganglion cells. My own investigations have been limited to the brain lobes, lateral nerve chords, and oesophageal nerves.

Before the appearance of M'Intosh's monograph ('73), no studies had been made upon the elements of the nervous system of the nemerteans. Since that time a considerable number of investigations have been published, only the more important of which will be specially mentioned here. Reference may be made to the papers of M'Intosh ('75, '76), Hubrecht ('74, '75, '79, '80, '87a, '87b), Mosely ('76), v. Kennel ('77), v. Graff ('79), Gulliver ('79), Dewoletzky ('80, '88), Salensky ('84, '86), Vogt and Yung ('85), Bateson ('86), Bürger ('88, '90a, '90b, '91a, '91b, '94a, '95), Haller ('89), Joubin ('90, '94), Dendy ('92), and Coe ('95a, '95b).

It is, however, v. Kennel, and more especially Hubrecht and Bürger, who have added most to our knowledge of the nervous system of the nemerteans. Hubrecht had especially studied the differentiation of the nervous system in its various layers, and had described the course of the larger nerves, etc.; he studied to less extent the cytological structure. Bürger considered not only the anatomy, but also the finer structure of the elements

of the nervous system. His more comprehensive papers, those to which reference will be made in the following pages, are '90b, '91b, '95; the latter paper really including the results of all his previous investigations, and furnishing also a number of new facts. Bürger first showed that all the ganglion cells of the nemerteans are unipolar and membraneless, and he divided the ganglion cells into four natural categories, which I follow him in adopting; he is also the discoverer of the colossal neurochord cells, and has been the first to recognize and distinguish the connective-tissue elements of the nervous system. This investigator has further been the first to apply the *intra vitam* methylene blue stain to this group, and by means of it has furnished most valuable contributions to the knowledge of the nervous system, especially with regard to the ganglion and nerve cells of the proboscis, and the course of the axis cylinders in the lateral chords and peripheral nerves. Though the results of my studies partly corroborate Bürger's conclusions, still in certain points I have reached views not in accord with his, especially in regard to the structure of the nerve tubules and of the dotted substance of the fibrous core, and of the genetic and structural relations of the neuroglia to the nervous elements.

My material was collected at Newport, where Dr. Alexander Agassiz had most kindly offered me a table in his private laboratory, and at Wood's Holl, where, through the kindness of Colonel MacDonald, I occupied a room in the Fish Commission Station. I would express my obligations to both these gentlemen. Colonel MacDonald's recent death was a sad shock to all who had known him personally or had experienced his generosity. *Lineus gesserensis* I collected in large numbers, it being abundant at both these localities; of *Cerebratulus lacteus*, however, I secured only a single specimen. In addition to these species I received a large *Lineus* from Norway, which I was unable to determine, and shall refer to simply as "*L. sp.*"[1]

[1] This undetermined species of *Lineus* had, after preservation in alcoholic sublimate, a diameter of about 3 mm., and was of a cylindrical form; the color, above a dark olive, lighter ventrally, with a fine, median, dorsal stripe of a yellowish color, and a similar stripe on each side of the body. I am indebted to my friend Dr. Fritz Schaudinn of the Berlin zoölogical laboratory for collecting it for me at Bergen.

The best fixative for the ganglion cells I have found to be corrosive sublimate in alcoholic solution (50% alcohol); addition of acetic acid to this solution is to be avoided. The best differentiating double stain is Ehrlich's or Delafield's haematoxylin, followed by eosin. The Ehrlich-Biondi stain does not furnish as sharp differentiations as the preceding. For following the course of the nerve tubules, preparations fixed for ½–1 hour in Hermann's fluid (platinum chl. + osmic acid + acetic acid) may be especially recommended; Flemming's fluid (chromic + osmic + acetic acid, in the stronger solution) is also valuable. Osmic acid alone, as well as Kleinberg's fluid (picrosulphuric acid), Lang's fluid, Perenyi's fluid (chromo-nitric acid), and all single solutions of chromic acid are not to be recommended.[1] The gold-chloride method of Apáthy ('91) might also be applied with good effect to the nemerteans, though I have not had opportunity to use it.

II. Ganglion Cells.

I follow Bürger's ('90b) precept in dividing these cells into: (1) the smallest sensory cells; (2) the medium-sized cells; (3) the large cells; and (4) the colossal neurochord cells (which are absent in *Lineus*). The cells of these different categories may be referred to respectively by the use of the roman numerals I, II, III, IV. Bürger's division of the ganglion cells I have found to be a very natural one, being based upon morphological differences as well as upon differences in regard to their relative positions in the central organs.

I can corroborate Bürger's ('90b, '91b) conclusions that all these cells are devoid of cell membranes, and that all are unipolar, — adopting his definition that a cell is unipolar when it has but one pole from which the cell processes depart, whether one or more processes are given off from the same pole. I have found only one process of the cell, namely, the true nerve tubule; and have never seen so-called "protoplasmic fibres" such as he has figured ('90b, Fig. 61 *g*, *h*). Haller's ('89) con-

[1] For more detailed results upon the action of different fixatives and stains, cf. Montgomery, '96b.

clusions that the nemertean ganglion cell is multipolar must either have been based upon the study of poor preparations or have been the result of preconceived views.

A. *Cell I.*

In *Lineus gesserensis* these smallest ganglion cells are usually densely massed together and of a shortened pyriform shape (Fig. 1). The nucleus is very large in proportion to the cell body, in fact nearly filling it; it is relatively very much larger, but varies less in size, than those of the other ganglion cells.[1] The nuclei vary from a spherical to an elongate-oval shape. Their chromatin is not limited to the periphery, but distributed in the form of nearly equally sized grains (of the number of ten or more in each nucleus), placed throughout the faintly staining nuclear sap; I have been unable to constate an achromatic network. In the nucleus one or two nucleoli may sometimes be found; these stain deeply with eosin, are of a spherical shape, and are larger than the chromatin masses. Sometimes an elongated "tail" of the nucleus penetrates for a short distance into the nerve tubule; but in none of the types of ganglion cells have I seen such branches of the nucleus as H. Schultze ('79) has described; it is to be noted that Leydig also never saw such structures. The cytoplasm usually stains very faintly, which is due to the excess of the hyaloplasmic vacuoles, these being enveloped by only a fine spongioplasmic meshwork.[2]

[1] Bürger has shown conclusively that these are sensory and not motor cells; and it would be interesting, in view of the relatively great size of their nuclei, to determine whether in other animal groups also the nucleus of the sensory is relatively greater than that of the motor cell; in other words, whether the size of the nucleus stands in relation to the function (sense, motion) of the ganglion cell.

[2] I shall employ in my descriptions of the cytoplasm the terms "hyaloplasm" and "spongioplasm" to denote respectively the homogeneous, unstaining, and the more or less granular staining constituents. Since Flemming ('82a) has by no means proved the fibrillar structure of protoplasm, I see no reason to adopt, as Rohde ('87) and Bürger ('90b) have done, his terms "paramitom" and "mitom." In a more recent paper ('92) Rohde has adopted Leydig's terms. The terms of Leydig, "hyaloplasm" and "spongioplasm," are more widely applicable, and thus preferable, since they express respectively the more fluid, homogeneous, and the more dense, more compound portions of the cytoplasm. I have never found a

Bürger ('90b, p. 107) describes a modification of these cells, "welche sich durch etwas grössere Kerne und lebhafteres Hervortreten des Zellplasmas von jener unterscheidet," and are situated around the cephalic clefts; but I have been unable to find any structural distinction between these two. However, two modifications of cells I may be distinguished as follows:

(a) A group of cells on the medio-dorsal side of the brain lobe, situated a little behind a frontal plane passing through the first ventral commissure. Their nuclei average larger, are as a rule more elongate-oval in form, and stain less deeply than the following.

(b) The greater number of the cells I on the dorsal brain lobe, *i.e.*, all with the exception of those in group (a); they have typically (though not always) spherical, deeply staining nuclei. But there is only a slight degree of difference between these two modifications, and intergradations are found between them.

In *Cerebratulus* these cells do not differ appreciably from those of *Lineus*, and occur in the same two modifications (Fig. 17 *a*, *b*).

B. *Cell II.*

Lineus gesserensis (Fig. 2).— These cells are usually more or less elongated, pear-shaped, with the greatest diameter proximally, becoming distally gradually more slender; seldom have they a shortened, oval form. The more or less centrally placed nucleus contains a relatively smaller amount of chromatin than that of I and III, and differs from the latter further in its elongate-oval, and not spherical, shape. One nucleolus (*n*) is sometimes found in it. The cytoplasm is of a coarsely vacuolar structure; sometimes the hyaloplasm fills the whole proximal portion of the cell as far as the nucleus. But a thin, peripheral layer (*Alv.*) of spongioplasm is always present, and a similar layer envelops the nucleus; these two layers, which may repre-

fibrillar structure of the spongioplasm of the ganglion cells. Further, these terms can be applied to denote the more fluid contents of the less fluid meshwork in describing a "honeycombed" structure of protoplasm (though Bütschli, '94, has endeavored to avoid the use of any such descriptive terms as might imply a difference between the substance of the meshwork and the substance contained within it).

sent alveolar layers (in the sense of Bütschli, '94), will be considered more in detail in treating of the ganglion cells III. In their distribution the cells II are limited to the ventral brain lobe and to the lateral chords, forming the greater part of the ganglion-cell layer of the latter. In their arrangement a radial grouping of a number of cells (thus giving rise to a cell cluster) around an opening in the inner neurilemma seems to be the rule; but the radial clusters are not as distinct, nor is there a symmetrical grouping of them in the lateral chords, as in *Cerebratulus*. Further, in *Lineus* II is not as distinct from III as in *Cerebratulus*, but these two types of cells closely approximate in appearance, so that a very small III may have a marked resemblance to a large II; but the form and structure of the nucleus usually serve to distinguish them.

In *Cerebratulus* the cells II (Fig. 18) differ quite noticeably from those of *Lineus*, in addition to their greater size. Thus the cytoplasm is usually denser, *i.e.*, there is a proportionately greater amount of spongioplasm, and a coarsely vacuolar structure is seldom found. The nucleus is usually oval and varies considerably in size; it contains a number of irregular chromatin masses, and one (sometimes two) larger, spherical nucleoli (*n*). The grouping of these cells into radial clusters, which Bürger ('90b) has shown to be characteristic for them, and which is much more pronounced in this genus than in *Lineus*, is due to the fact that the nerve tubules of a considerable number (approximately fifteen or more) of neighboring cells converge together and penetrate in a bundle through a single opening of the inner neurilemma to reach the fibrous core (Fig. 18). On a section, by which only a few of the cells of a cluster are cut, the arrangement is fan-shaped (Fig. 25, *Cl. II*). This grouping is especially characteristic for II, though sometimes cells I present this arrangement to a limited extent.

These cells occur only in the ventral brain lobes and in the lateral chords, as shown by Bürger ('90b). But their symmetrical arrangement in the lateral chords in *Cerebratulus*, a point hitherto unnoticed, is worthy of mention. In each lateral nerve chord, namely, the radial cell clusters occur as in the ventral lobe, but with a certain regularity of distribution which is not

expressed there. On a transverse section of a lateral chord one finds, both on the dorsal and on the ventral side (ganglion cells are absent on the median and lateral aspects, as stated by Coe, '95a), a right and a left cluster of these cells (Fig. 25). For each radial group is one opening in the inner neurilemma, serving for the transmission of its bundle of nerve tubules; the respective openings of two neighboring clusters are separated from one another by a distance equivalent to the greatest diameter of a cluster. Accordingly, an anterior opening is separated from the next following posterior opening, and an opening on the right hand from one on the left. Thus, both on the dorsal as well as on the ventral side of a lateral chord, there is a single linear series of cell clusters on the right, as well as such a series on the left; and, consequently, also two corresponding series of openings in the inner neurilemma. It is the rule, further, that the cell clusters on the right hand alternate with those on the left, on each side of the chord, and *pari passu* their respective openings on the right with those on the left; this alternation becomes more marked towards the posterior end of the chord, where, owing to the decreasing diameter of the latter, the distances between the successive clusters become relatively greater. So it is seen in Fig. 25, that on either side of the chord the cluster on the right does not lie in the same plane as that on the left. In other words, on either the dorsal or the ventral side of each lateral chord, the clusters of ganglion cells II and their respective openings in the inner neurilemma (these serving for the passage of their respective bundles of nerve tubules into the fibrous core) are symmetrically and bilaterally arranged. But this arrangement does not represent a true bilaterality, since the cell clusters on the right are not paired with those on the left, but rather alternate with them. To adopt the terminology of Bateson ('94), the relation of the two lateral nerve chords to one another would constitute a "major symmetry" (the one being a mirror image of the other); while that of the right and left sides of each chord would represent a "minor symmetry." Fig. 26 represents a diagrammatic longitudinal section (in the horizontal plane), through either the dorsal or ventral side of one

nerve chord: *Cl. II*, the cell clusters; *P*, their respective openings in the inner neurilemma; *x–x*, the imaginary median plane of symmetry.

C. *Cell III.*

a. *Lineus.* — These cells (Figs. 3–16) are of an elongated pyriform shape, largest and rounded proximally, seldom nearly spherical. It may be noted that while the cell bodies vary considerably in size, their nuclei remain of nearly uniform dimensions.

The nucleus (*N*) seldom lies in the proximal portion of the cell, but usually near the center, or even in the constricted, distal portion; this would show that it, as well as the nucleus of I (*v. supra*), stands in as close a physiological connection with the substance of the nerve tubule, as with the cytoplasm of the cell body itself; this fact might well be borne in mind by those who suppose the vitality of the cell process to be more or less distinct from that of the cell. The nucleus is usually spherical, and larger than those of any other somatic tissues, except a few of those of the outer neuroglia cells (Montgomery, '97). Its chromatin is more or less regularly distributed in granular form, but occasionally produces a thick peripheral layer, from which a few chromatic fibres pass towards the nucleolus. Such reticulation of the chromatin is especially discernible on preparations fixed with a fluid containing OsO_4; while the action of corrosive sublimate obliterates to a great extent whatever reticulation may exist, and imparts a granular appearance to the chromatin. One nucleolus (*n*) is always present; occasionally there are two, and then of unequal size; the single nucleolus is most frequently centrally situated, and then is enveloped by a sheath of chromatin.

The cytoplasm is, as a rule, coarsely vacuolar (vesicular), especially so towards the distal pole. A thin peripheral layer of finely granular cytoplasm is always present (Figs. 3–16, *Alv.*); this layer might compensate physiologically for the absence of a cell membrane, as has been suggested by Leydig ('64). Bürger ('90b), who has also noticed this layer, described it as more coarsely granular than the rest of the cytoplasm. This

compels us to refer briefly to the methods of fixation with which this investigator's material had been preserved. Part of it was fixed with weak chromic acid solutions, which are not to be recommended. He states, further, that "Die Behandlung derjenigen [*Cerebratulus marginatus*, *Langia*] aus der Zoologischen Station in Neapel ist mir unbekannt"; the fixative used for these, I presume, had been sublimate in alcoholic solution — a method much employed at the Naples laboratory. Thus Bürger had used neither Hermann's nor Flemming's fixation fluids, both of which demonstrate the fine-grained structure of the peripheral spongioplasmic layer much more clearly than does sublimate, which generally produces a fusion of the microsomes into larger granules.[1] A similar fine-grained layer of spongioplasm may almost always be found enveloping the nucleus.

As has been already remarked, the greater part of the cytoplasm is coarsely vacuolar, in that staining, non-fibrillar, spongioplasmic meshes envelop various-sized, unstaining, structureless vacuoles of hyaloplasm, which in life is probably fluid. There does not seem to be any concentric or other regular arrangement of these vacuoles around the nucleus, though Bürger has given two figures exhibiting such a distribution ('90b, Fig. 61 *d*, '91b, Fig. 18 *a*, the latter from life); this author considers that such an arrangement of the hyaloplasmic vacuoles is the natural one, and that preparations which show no such arrangement are artifacts. Now while I have never observed any such regular arrangement, I would not maintain that it does not occur, but merely that it must be very infrequent; but, on the other hand, the irregular grouping of vacuoles of unequal size must not be regarded as artificial, but as normal, since it is found after the use of the most diverse fixing reagents. I consider

[1] This peripheral, fine-grained layer of the cell had been seen by Leydig ('64), who quite correctly showed that it does not correspond to a cell membrane. But H. Schultze ('79), who found it to be continued along the axis cylinder, considered it a true cell membrane. Nansen ('87), as I understand him, was unable to decide whether the ganglion cells possess true membranes or only such as are formed by the encircling neuroglia fibres. Rohde ('90a, '92) supposed all the spongioplasm of the ganglion cells of *Polychaeta* and *Hirudinea* to be fibrillar, and composed of neuroglia fibres which had penetrated the cell.

the various groupings of these vacuoles within the cell as corresponding to the different physiological states of the latter. Rarely does the spongioplasm preponderate quantitatively throughout the cell, though this is the rule for the ganglion cells of the commissures of the oesophageal nerves.

To recapitulate : a fine-grained layer of spongioplasm forms the peripheral boundary of the cell, and a second similar layer envelops the nucleus. The remainder of the cytoplasm is usually coarsely vacuolar, especially distally, these hyaloplasmic vacuoles being of unequal size and without appreciable arrangement into concentric layers around the nucleus. Now the fine-grained layers around the periphery of the cell and encircling the nucleus I would consider *alveolar layers*, in the sense of Bütschli ('94), their microsomes thereby representing the nodal points of an alveolar meshwork, and the unstaining, hyaloplasmic spaces between these supposed nodal points the more fluid contents of the meshes ("Waben"). Whether similar alveolar layers also bound the larger vacuoles I have been unable to determine, even after careful investigation. According to Bütschli's conclusions, these larger vacuoles would not correspond to individual meshes, since they exceed the size of a primitive mesh ("Wabe," which, according to his studies, rarely exceeds 1μ in diameter, although occasionally it may measure 8μ). On the whole, however, the cytoplasmic structure of cells II and III, in both *Lineus* and *Cerebratulus*, and of IV in the latter (I being too small for investigation), gives the impression of a honeycombed meshwork, as described by Bütschli for the cytoplasm of especially vacuolar Protozoa, such as *Actinosphaerium*.[1] Even the presence of the two alveolar layers described by me would alone render it probable that this is the structure of these ganglion cells, since Bütschli has shown pretty clearly that the presence of such alveolar layers is only to be explained on the presupposition of a honeycombed meshwork.

[1] Schaudinn, in a number of papers upon Rhizopoda published in the last three years, has confirmed Bütschli's results on the structure of the protoplasm of the Protozoa. I would express the view reservedly that the honeycombed meshwork probably represents the structure of the less differentiated protoplasms, and is the more primitive structure; but that this structure may become radically changed in more differentiated cells.

In none of the nemertean ganglion cells are neuroglia fibres present; there seems to be no penetration of these elements into the cells, and the spongioplasm of the latter is certainly not produced by them.

There remain to be described certain bodies occurring in the cytoplasm of III in *L. gesserensis* and *L. sp.*, these bodies being restricted to these cells in this genus and absent in all ganglion cells of *Cerebratulus* (*Gr.* in Figs. 3, 6, 8, 9, 11, 12, 14, 15). These are frequently larger than the nucleolus and of a spherical or oval, regular shape, and are not refractive. After the use of a double stain, they stain usually with eosin, sometimes with haematoxylin, but always more intensely than the surrounding cytoplasm, though seldom as deeply as the nucleolus. Structurally they are homogeneous, with a peripheral membrane, which may be scarcely discernible, or in other cases of considerable thickness; this membrane always stains more intensely than the enclosed portion, and forms a boundary against the surrounding cytoplasm. These bodies do not occur in all cells, but only in about one-sixth of the total number; when they are present, it may be but a single one, more frequently four or five, apparently never more than about fifteen. There is also no regularity in their distribution, such as a concentric or radial arrangement, and in the same cell they are usually of various sizes and of different staining power; sometimes, however, two of equal size and similar staining power lie in contact (Figs. 11, 16), so that a distribution in pairs seems to be not infrequent. They are always absent in the nerve tubule. To these cytoplasmic bodies may be applied the term *chromophilic corpuscles*, to distinguish them from the *chromophilic granules* in the ganglion cells of other animals. These corpuscles are certainly not artifacts, since they become preserved equally well with Hermann's and Flemming's fluid and with sublimate (aqueous with or without acetic acid, alcoholic solutions). Further, they cannot be considered parasitic organisms, since they are found only in these cells in *Lineus*, and not in the other ganglion cells nor in the surrounding neuroglia; they are also not pathological, since I found them in all (about a dozen) of the individuals studied. Again, they cannot be regarded as aggregates of

nutritive substances which the cell has taken up from without, since they are never found within the nucleus.[1] I conclude that the chromophilic corpuscles are metamorphosed portions of the cytoplasm produced by a dense welding together of the microsomes of the latter at different points in the cell body; this would account for their power to stain deeply, and their homogeneous appearance. Again, some stain scarcely more deeply than the surrounding cytoplasm, and their bounding membranes are scarcely appreciable, while others stain intensely, and are provided with relatively thick limiting membranes; and all intergradations between these two stages occur. These facts are sufficient to show that the chromophilic corpuscles of *Lineus* are differentiated portions of the cytoplasm of the cell, the process of differentiation consisting in a compression or fusing together of the primitive microsomes. Analogical metabolic cytoplasmic changes are found, *e.g.*, in the formation of yolk particles in the egg cell of *Stichostemma* (Montgomery, '95).

Corpuscles, somewhat similar to these, I find described by Dehler ('95) for the sympathetic ganglion cells of the frog: "Diese Schollen . . . haben eine ziemlich unregelmässige Gestalt: von einer körnchenartigen Zusammensetzung an findet man sie bis zur Gestalt von flachen Dreiecken mit breiter Basis; auch grosse Schollen, die aus kleineren lose zusammengesetzt erscheinen, sind vorhanden; sie sind nicht kugelig und haben niemals scharfe Ecken, so dass der Ausdruck "Scholle" der einzig passende zu sein scheint. . . . Manchmal finden sich in kleineren Zellen wenig oder keine chromatischen Schollen; dafür ist dann die Zelle diffus und intensiver gefärbt. Flesch nennt dieses Verhalten Chromophilie. Ich erkläre mir das so, dass in solchen Fällen die chromatische Substanz nicht wie zu Schollen geronnen, sondern diffus in die achromatische Substanz verteilt ist; wahrscheinlich ist sie in den kleineren Zellen in derselben oder fast derselben Menge vorhanden, nur gleichmässiger verteilt wie in den grossen." Dehler finds these "Schollen," which differ from the corpuscles of *Lineus* in their

[1] Cf. Korschelt's studies, "Zur Morphologie des Zellkernes," and the observations of mine upon nutritive processes in mesenchym cells ('97).

irregular form and greater size and number, distributed in concentric circles around the centrosome (not around the nucleus), the largest "Schollen" being placed peripherally.[1]

Three years previous to Dehler's researches, Vas ('92) studied the chromophilic bodies, which he termed "chromatin," in sympathetic ganglion cells of man, horse, dog, and rabbit; they are, in these forms, smaller than those observed by Dehler in the frog. Vas did not notice a concentric arrangement, but found the largest bodies peripherally situated. He makes the interesting observation that the granules are absent in the cells of the human foetus, and first appear after birth.

The *chromophilic granules* of ganglion cells, such as have been described (mainly for vertebrates) by Flesch ('86a, b), H. Virchow ('88), Nissl ('94a, b, and previous papers), de Quervain ('93), van Gieson, Lenhossék ('95a), Pflücke ('95), and others have but little resemblance to the chromophilic corpuscles of *Lineus*. For the "granules" are much smaller, usually spindle-shaped, and are arranged concentrically around the nucleus. One point of similarity may be referred to, however: the chromophilic granules as well as the corpuscles occur only in the cytoplasm of the cell, never in the nucleus nor in the axis cylinder. Lenhossék ('95a), who has studied the chromophilic granules in the multipolar spinal ganglion cells of *Homo*, *Bos*, and *Lepus*, states: "Es wäre allerdings möglich, dass sie, wie de Quervain ('93) vermutet, durchgehends Komplexe von feineren Körnchen sind, aber ein solcher diskontinuierlicher Aufbau lässt sich nur an einer kleinen Anzahl von Schollen nachweisen"; and notes that although the chromophilic granules do not enter the axis cylinder, they nevertheless penetrate the dendritic processes for a short distance.

The recent paper by Pflücke ('95), based upon studies on ganglion cells of *Lumbricus* and Mollusca (fixed with sublimate), describes as follows the arrangement of the chromophilic granules: "Der Zellleib der Nervenzelle besteht aus varikösen

[1] Centrosomes were discovered for the first time in ganglion cells in the frog by Lenhossék ('95b) and Dehler ('95), independently of one another; by the former in the spinal ganglion cells, by the latter in those of the sympathetic ganglia. I have not found centrosomes in the ganglion cells of the nemerteans.

Fibrillen, die sich im Umkreis des Kernes, unter Bildung zahlreicher Queranastomosen in ein Netzwerk auflösen. Die Varikösitäten sind eine besondere Eigenschaft der Plasmafibrillen, da sie denjenigen des Achsencylinders fehlen oder doch nicht durch die Färbung nachzuweisen sind. . . . Die Kernmembran besitzt knötchenartige Verdickungen von gleicher Beschaffenheit, wie diejenigen der Plasmafibrillen. Diese Knötchen bilden die Vereinigungspunkte der sowohl vom Plasma, als auch vom Kerngerüst ausgehenden Endfäserchen" (p. 538). In justice to that excellent observer Leydig must be stated that he was the first to figure such varicose, concentric fibrils ('85). What Pflücke describes as varicosities of supposed concentric cytoplasmic fibrils (which he states compose the "primitive fibrils" of the axis cylinder) correspond to the chromophilic granules of other authors. A certain similarity is to be noticed between his conclusions and those of Nansen ('87). Thus Nansen, although he had not seen the chromophilic granules, described the primitive tubes of the axis cylinder as encircling the nucleus concentrically; the concentric fibrils of Pflücke might correspond to the sheaths of such primitive tubes, or *vice versa*. Pflücke's paper reopens up a field which certainly deserves reinvestigation.

In the ganglion cells of *Unio*, H. Schultze ('79) found "grössere, doppeltcontourirte, myelinähnliche Tropfen," which became browned by osmic acid; if these really contain myelin, which is doubtful, they would correspond neither to the chromophilic granules nor to the chromophilic corpuscles.

b. *Cerebratulus.* — The cells III in this genus differ slightly from those of *Lineus* and are considerably larger (Figs. 19–21, 29, *III*); they resemble closely those described by Bürger ('90 b) for *C. marginatus*. They are usually flask-shaped, swollen and rounded proximally, distally tapering to the axis-cylinder pole; sometimes even retort-shaped, ovoid, or nearly spherical; in the lateral chords they may be even angular in outline.

The nucleus (Fig. 22 *a–e*) is usually proximal or central in position, seldom distal (this being the frequent position in *Lineus*). It is usually nearly spherical or spherico-oval, sometimes kidney-shaped; but I have never seen one of a horse-

shoe shape, as found by Bürger ('90b, Fig. 61 *b*, *c*). Usually a coarse-grained peripheral layer of chromatin (*Chr.*) is present, each of these larger grains being apparently composed of finer microsomes ; and centrally, fine-grained chromatin is distributed in the nuclear sap. In only a few nuclei were evidences of a delicate achromatic reticulation to be found. Occasionally the chromatic granules are limited entirely to the periphery, the central portion of the nucleus containing only nuclear sap ; on the other hand, sometimes the whole nucleus appears to be nearly filled with chromatin. The different ways in which the chromatin may be distributed probably correspond to different physiological states of the nucleus, and cannot be considered artifacts, since the same differences are found after the use of various reagents.

Each nucleus contains one large spherical nucleolus (*n*), which is usually centrally placed, rarely peripherally, and never in close contact with the nuclear membrane ; this latter position is typical for the nucleoli of cells IV. A layer of chromatin granules usually surrounds the nucleolus, and from this layer fibres radiate towards the peripheral masses of chromatin. Not infrequently two nucleoli are present, which are then usually of different size and placed at varying distances from each other ; but in only one case did I find the two nucleoli stained differently (Fig. 22 *b*). I never found more than two nucleoli, and never saw evidences of nuclear division, though I had paid particular attention to these points.

Bürger states ('90b, p. 113) : "Bei I habe ich immer mehrere [nucleoli] beobachtet," which shows that he had confounded chromatin masses with nucleoli. By the term *nucleolus* I understand, in accord with most cytologists, a body contained within the nucleus, usually of a spherical form, which stains intensely with eosin (after the use of the double stain, haematoxylin + eosin). It should not be confused with the irregular masses of chromatin, which stain with haematoxylin, and each of which is composed of finer microsomes. Bürger has also described for *C. marginatus* and *Langia* slightly staining, but little refractive ("von mattem Glanz") globules (Bläschen) continued within the nucleus. Thus ('90b, p. 113) :

"Dasselbe liegt fast immer dicht neben dem Nucleolus oder umfasst selbst denselben. Zuweilen sah ich auch viele kleine Bläschen um den Nucleolus gelagert oder an der Membran des Kernes kranzförmig angeordnet, in einem besonders grossen lag alsdann der eigentliche Nucleolus. War der Kern, d. h. der gefärbte Bestandtheil desselben nierenförmig oder ähnlich gestaltet, so umschloss er das Bläschen." In *Langia* he found the "Bläschen" of an enormous size, nearly filling the nucleus. Rhumbler ('93) has reproduced four of Bürger's figures showing these Bläschen, in support of his theory of the formation of the "Binnenkörper." Such structures as these "Bläschen" of Bürger are positively absent in the nuclei of all ganglion cells which I have examined. Bürger's "Bläschen," such as he described them, can be only artifacts, that is, artificially centralized portions of the fluid nuclear sap, and their apparent globular form is due to an optical illusion. In other words, I would regard them, not as distinctly defined spherical bodies, but merely as portions of the nuclear sap which fills all interstices between the chromatin granules. This is shown by my Fig. 22 *b*, *e*. Whenever the chromatin forms a sharply bounded peripheral layer and the central portion of the nucleus is filled entirely with nuclear sap, as is not infrequent, it is only an optical illusion which could lead to the assumption that a large, unstaining globule nearly fills the nucleus. It has been necessary to refute Bürger's observations on this point, since he has described a nuclear structure, which would be nearly unique in the Metazoa, had it been accurately portrayed. Rohde ('90a) described similar globules in the nuclei of the ganglion cells of Polychaetes.

I have found only one cell containing two nuclei (Fig. 21) among the hundreds of ganglion cells examined. These nuclei differed both in size and structure, which moots the question whether this particular cell should not be considered a fusion of two cells, rather than a cell whose nucleus had divided into two, while the cell body remained intact. For in the latter case it would be probable that both nuclei should exhibit the same size and structure.

In *Cerebratulus* the cytoplasm of cells III is not, as a rule, as vesicular as in *Lineus*, but both hyalo- and spongioplasmic constituents are pretty evenly distributed. In some cases the hyaloplasm nearly fills the whole cell body, though even in such cells a peripheral, fine-grained, spongioplasmic layer envelops the cell, and a similar layer surrounds the nucleus. Occasionally, also, I found a large, flattened vacuole, resembling in shape a concavo-convex lens, situated between the nucleus and the proximal end of the cell ; the structure of the cytoplasm in the rest of the cell being finely granular.

The cells of the commissures of the oesophageal nerves are a modification of the cells III of the brain, from which they differ in their inferior size and denser cytoplasm (Fig. 20). In the lateral chords, two or three cells III often surround a cell IV, and such enveloping cells are often difficult to distinguish in structure from the enclosed IV (Fig. 29).

Bürger ('90b) has already shown that the cells III occur both in the dorsal and ventral lobes of the brain and in the lateral chords. It may be noted that in *Lineus* they are comparatively more numerous on the periphery of the ventral lobe than in *Cerebratulus ;* while in the latter genus they are relatively more abundant on the medial side of the dorsal lobe than in *Lineus*. Only in *L. sp.* do I find a few cells III on the lateral aspect of the lateral nerve chord ; this, as well as the median side of the chords, being always devoid of ganglion cells in *Lineus gesserensis* and *Cerebratulus*.

D. *Cell IV* (*Cerebratulus*).

These colossal cells, termed by their discoverer, Bürger ('94b), "neurochord cells," on account of their similarity to the neurochord cells of other forms, have been found by him to be limited to the genera *Cerebratulus*, *Langia*, *Drepanophorus*, and *Prosadenoporus*. They are absent in *Lineus*, but I have found them in *Cerebratulus lacteus*. Coe ('95a) did not observe them in the latter species, which is a strange oversight, since they are the largest cells of this nemertean, with the exception of the ova ; however, it is possible that this author classed them together with cells III.

a. *Distribution.* — In *Cerebratulus marginatus* and *Langia formosa*, Bürger found one pair of these cells in the ventral lobe of the brain, and a considerable number placed irregularly along the lateral nerve chords. In *Drepanophorus* and *Prosadenoporus* he found only one pair, situated in the brain, and none along the lateral chords.

In *C. lacteus* I have made a more detailed investigation of their distribution, based upon a study of several series of transverse sections (of about 5μ thickness) through an immature individual of approximately 6 in. in length. In this way about four-fifths of the worm was sectioned. Since it was not my intention to determine the total number of neurochord cells in the body, — a number which probably varies in different individuals, — but rather to determine their proportionate number in the two lateral chords and on the dorsal and ventral sides of each chord, it would have been an unnecessary consumption of time to cut the whole animal into thin sections of 5μ thickness. Even had this been done, the total number of cells could have been given only approximately, since quite a number of cells occur which are structurally intermediate between III and IV, putting the calculator into the quandary of not knowing whether to count them in or whether to leave them out ; and whichever way he should determine upon, he could not be certain of the correctness of his resulting figures.

Three pairs of neurochord cells are present in the ventral lobes of the brain (three cells in each lobe). The cells of the first pair (Fig. 24, *C. IV*) lie close beneath the dorsal blood vessel (*D. V.*), on the medial sides of the ventral brain lobes, in a frontal plane which cuts the oesophageal nerves about the point where they begin to pass out of the fibrous core of the lobes. Both cells lie in the same frontal plane. This was the only pair found by Bürger in the brain of *C. marginatus* and *Langia*. The second pair of cells do not lie in one and the same plane : the first cell lies on the right hand, one section behind the first pair of cells, the second cell lies on the left hand, five sections behind the first pair of cells; thus the cells of the second pair are three sections apart. The third pair of cells are placed two sections behind the most posterior cell of

the second pair (six sections behind the first pair); both cells of the third pair, just as those of the first, lie in the same frontal plane. These three pairs lie in nearly the same horizontal plane, and the distance separating the cells of the first pair is about equal to that between the cells of the third pair; but the space between the cells of the second pair is greater, since they are three sections apart.

Behind the brain no neurochord cells are found in the lateral nerve chords until the anterior region of the posterior intestine is reached, being absent in the chords in the oesophageal region of the body, as noticed by Bürger ('90b); in *C. lacteus* this is a distance of several centimeters.

Counting from before backwards, I find the first neurochord cell of the chords just behind the oesophageal region on the ventral side of the left chord; the second on the dorsal side of the right chord; the third on the ventral side of the left chord; the fourth on the dorsal side of the same chord; the fifth on the ventral side of the same chord; the sixth and seventh on the dorsal side of the right chord; the eighth on the dorsal side of the left chord; the ninth and tenth on the same section, on the dorsal sides of the right and left chords respectively. With the exception of the last two, the cells enumerated are separated (in the longitudinal axis) by considerable, though varying, distances. The sequence of the first ten cells of the lateral chords has been given as an example of their irregularity of arrangement. It is unnecessary to reproduce here the distributive sequence of the approximately 159 cells whose position I have determined, and it will be sufficient simply to state the general results of this study of their arrangement. The following table gives, for the four-fifths of the worm sectioned, the numbers of these cells in both chords, and of each side of each chord, all cells which are not typical neurochord cells being excluded:

	Right Chord.		Left Chord.	
	Dorsal	Ventral	Dorsal	Ventral
	68	16	55	20
Totals	84		75	

These figures show: (1) that the number is not equal in both chords, and (2) that there are about three or four times as many cells on the dorsal sides of the chords as there are on their ventral sides.

The neurochord cells are absent in the oesophageal region, and also in the caudicle; thus both ends of the lateral chords are devoid of them.[1] They increase in number from the anterior to the posterior end of the chord, and, in which I can also corroborate Bürger's observations, only dorsally situated cells occur in the region just anterior to the caudicle. They are always situated medially, close to the circular musculature, as Bürger states, but are frequently more or less lateral in position. The cells in the left chord are seldom paired with those in the right, though it is not infrequent, through a short extent, for those of the one to alternate with those of the other. But it is more usual for three or four to succeed one another in the one chord, while the corresponding portion of the opposite chord is devoid of cells, this order being reversed further on. Then, too, in the same chord dorsal and ventral cells do not alternate, and seldom occur together on one section (I found only two sections of a ventral chord, each of which contained a dorsal and a ventral cell); similarly, two cells in the same section, both on the dorsal side of a chord, I found only twice, and have figured one of these cases (Fig. 32). With these exceptions, the neurochord cells were separated, as a rule, by considerable; though irregular, distances, so that in a series of forty consecutive sections from the middle of the body, seldom were more than six neurochord cells to be observed in both nerve chords.

The only apparent regularity in their distribution is that, in certain portions of the chord at least, zones may be distinguished in which they follow one another in comparatively close sequence, alternating with areas (of approximately equal extent) where they are much less abundant. These alternating areas

[1] I have shown previously ('97) that in the caudicle the fibrous core of the lateral chord is directly enveloped, on all sides except the median, by a mass of true mesenchym cells, and that no ganglion cells accompany the chord in this region.

do not correspond to the number or position of the lateral nerves ("spinal" nerves) of the lateral chords. It would be important to determine whether the sequence of such zones corresponds, for instance, to the metamerism of the gonads; since my specimen was immature with undeveloped gonads, I could not determine this point. If such a regular metamerism of the areas could be proved, then the lateral chords of the nemerteans — in which no segmental localization of ganglion cells producing ganglia has as yet been shown — could be considered as in the inceptive stage of producing such ganglionic localizations. Unfortunately, however, the determination of this point, which has a certain phylogenetic importance, would necessitate the preparation of many thousand sections, since the adult worm should furnish the basis for such a study. But it might more easily be investigated on smaller species of the genus.

b. *Structure.* — The structure of the giant ganglion cells IV of *Cerebratulus* (Figs. 27–29, 32) has much resemblance to that of cells III of the same species, though there are certain differences which may usually serve to distinguish them.

The nucleus (Fig. 31 *a–e*) may be nearly spherical, but is more frequently spherico-oval. It usually has a proximal position within the cell, close to the cell membrane, is seldom central, and never distal in position. In it small masses or granules of chromatin (*Chr.*) of adequal size are arranged peripherally on the inner surface of the well-marked nuclear membrane; and these do not form a continuous layer, as is frequently the case in the nucleus of III, but are placed at more or less regular distances apart. The central portion of the nucleus, which Bürger ('90b, cf. my remarks on the nucleus of III) has erroneously supposed to be filled with a "Bläschen," is mainly filled with the nuclear sap; this sap, in contrast to that of the other ganglion cells, stains faintly with haematoxylin. In it vestiges of a finely granular, achromatic reticulum (meshwork ?) may be observed (*Achr.*). A thin mass of chromatin envelops the nucleolus (*n*). The latter is never absent, is of large size, and almost always peripherally situated; it has thus the same position in the nucleus as the latter has in the cell.

I have found only three or four cells containing two nucleoli apiece, and in each case the latter were unequal in size; these cells were placed about the middle of the lateral chords. Thus, it is not the case in *C. lacteus* that "die Zellen im hintersten Ende der Seitenstämme besitzen . . . häufig zwei gleich grosse Nucleoli," as Bürger ('90b, p. 117) has stated for *C. marginatus;* on the contrary, I found the most posterior cells to contain only one nucleolus. Nor yet, in the species described here, are the nucleoli of the first pair of neurochord cells in the brain directed towards one another.

The cell (Figs. 27–29, 31) is usually of a shortened, pyriform shape, occasionally nearly spherical, or again elongated (this is the case with the first pair in the brain). The cell, together with the thickened, proximal portion of its nerve tubule, often resembles an Italian wine-flask. As a rule, though not always, these cells are much larger than III.

The cytoplasm is, especially distally, coarsely vacuolar, more so than in any other ganglion cell; this gives the cell much the same appearance as a slime-producing gland cell. It is characteristic for the neurochord cells that the hyaloplasm (*Hl. Pl.*) usually stains slightly with haematoxylin. There is always a fine-grained, peripheral, spongioplasmic, supposedly alveolar layer, and a similar layer immediately invests the nucleus (*Alv.*). The mass of cytoplasm is never finely granular throughout, such as is sometimes the case in III, but the distal portion at least is always vacuolar; and as in III, there is no special linear, radial, or concentric arrangement of the hyaloplasmic vacuoles. In IV also the general structure of the cytoplasm apparently corresponds to that of a honeycombed meshwork (Bütschli, '94).

E. *Comparison of the Ganglion Cells.*

In *Lineus* the ganglion cells are not so strongly differentiated, *i.e.*, represent a lower stage of development than in *Cerebratulus.* Thus in the former genus there are no neurochord cells, and the cells II and III approximate much more closely than in the latter.

In both these genera cell I differs markedly from the others in (1) its small size, (2) the relatively great size of its nucleus, and its large proportion of chromatin (Figs. 1, 17). Bürger ('90b, '91b) has shown these cells to be sensory in function, which their position, etc., renders very probable.

The cell III in structure closely resembles II, especially in *Lineus*. Cell III (Figs. 3–16, 19–21, 29, *III*) is structurally intermediate between II and IV, and even grades into them; while II (Figs. 2, 18) and IV (Figs. 27–29, 32) never intergrade, but are always to be sharply distinguished.

III resembles II in: (1) its usually elongated form, (2) its more or less marked radial arrangement around the fibrous core (in *Lineus* it is occasionally even grouped in radial clusters), (3) in the usually central or distal position of the nucleus.

III resembles IV in: (1) its size (very large examples being fully as large as small specimens of IV), and (2) in the usually peripheral distribution of the chromatin within the nucleus. Some cells intermediate in structure between III and IV occur in the lateral chords. Thus the cell reproduced in Fig. 23 resembles IV in the coarse vacuolization of its cytoplasm, and in the peripheral, proximal position of its nucleus; but it is similar to III in its small size, the arrangement of the chromatin, and the position of the nucleolus within the nucleus.

Since it is the rule in most of the higher Metazoa that the larger ganglion cells are motor cells, and since III, which is the largest cell in *Lineus* and next to the largest in *Cerebratulus*, shows structural resemblances to II on the one hand, and to IV on the other, I would conclude that these three types of cells are related in function, as well as in structure, and that they are probably all motor cells. Bürger ('91b, '95) has already produced good evidence to show that IV cannot be sensory cells, since he found that their axis cylinders do not pass out of the lateral chords; and having found that they occur only in genera which have the power of swimming, he makes the suggestion that they are directly concerned in this form of motion. I give the following diagram to represent my views on the morphological and physiological, though not necessarily histo-

genetic, relationships of the four types of nemertean ganglion cells:

Sensory cell } I III < II, IV } Motor cells.

It may be noted that in the nemerteans the size of the ganglion cells stands, as a rule, in proportion to the size of the body. Thus *Cerebratulus lacteus*, the largest species examined here, has larger cells than has *Lineus sp.;* and the latter species has larger cells than the still smaller *L. gesserensis*. And I find in the *Metanemertini* also that *Amphiporus glutinosus* and *A. virescens* have larger cells than the smaller species, *Tetrastemma catenulatum*, *T. vermiculum*, and *Stichostemma eilhardi*. An exception to this rule is *Carinella annulata*, with smaller cells than *Lineus sp.;* but *Carinella* is a most primitive form, in which the ganglion cells and the whole nervous system have become but little differentiated.

III. Nerve Tubules.

A. *Cell III.*

In *Lineus gesserensis* I have studied the nerve tubules on thin sections (*c.* 3μ thickness) on material prepared with sublimate (aqueous, alcoholic solutions), Flemming's and Hermann's fixatives, and treated with different stains.

Each ganglion cell has one axis-cylinder process, and I have been unable to find dendritic (so-called "protoplasmic," or "Deiter's") processes.

The cytoplasm of the ganglion cell (Figs. 3–14) is usually most vacuolar distally, where the cell tapers towards the axis-cylinder pole. But even here the mass of cytoplasm is always bounded by a peripheral, fine-grained layer (*Alv.*). This peripheral layer is directly prolonged along the axis cylinder, and produces the very fine spongioplasmic sheath of the latter (Figs. 3–14, *Alv.*). This sheath is, with the exception of the sheath of Schwann (to be described later, cf. "Neuroglia"), the only staining portion of the nerve tubule, and also the only part in which a fine-grained structure can be discerned. This fine

outer spongioplasmic sheath of the nerve tubule immediately envelops the axis cylinder, which cylinder composes the greater part of the tubule, and is a direct continuation into the latter of the hyaloplasm (*Hl. Pl.*) of the cell. Thus the axis-cylinder process is a *nerve tubule* and not a *nerve fibril*, since by the term "fibril" is implied a very fine thread of more or less dense (*i.e.*, non-fluid) composition, which is also of equal density in its whole diameter. In short, the typical structure of the ganglion-cell process in *Lineus* is that of a nerve tubule, consisting of a very fine-staining, fine-grained, spongioplasmic sheath, which envelops the unstaining, hyaloplasmic axis cylinder.[1] The hyaloplasmic axis cylinder never stains with any of the methods employed by me; an exception is found in the *intra vitam* methylene blue staining; thus Apáthy's ('91) investigations show that the axis cylinder (his "Primitivfibrille") stains as well as its sheath; and since Bürger's ('91b) Fig. 18 *a* shows that both hyalo- and spongioplasm stain within the ganglion cell, it is allowable to conclude that both the axis cylinder and its sheath become stained with methylene blue. I might also refer to my Fig. 41, from a preparation of *Tetrastemma* fixed with sublimate and afterwards stained with aq. sol. of methylene blue; here the structure lettered *N. T. ?*, which probably represents a nerve tube (*i.e.*, a bundle of nerve tubules), has become deeply stained throughout with the methylene blue. Further, the axis cylinder is always non-refractive and homogeneous, and does not contain longitudinal "primitive fibrils," nor other fibrous or granular structures; nor yet have I found any evidences of a meshwork structure, as described by Bütschli ('94). The substance of the axis cylinder is always homogeneous and structureless, and is identical with the hyaloplasm of the cell. This hyaloplasm must be a fluid, or at least a viscid, substance in life; but it certainly cannot be dense, since in that case we would expect to find it refractive, and to

[1] The very fine membrane around the axis cylinder in vertebrate nerve fibres, as figured by Koelliker ('89), might correspond to such a spongioplasmic sheath as that described by me in nemerteans. The "primitive nerve fibrils," such as have been described for a large number of forms, lie within the substance of the axis cylinder.

find it showing evidences of a finer structure. It is strange that Apáthy ('91), whose description of the axis cylinder (his "leitende Primitivfibrille") of the *Hirudinea* closely corresponds to mine of the *Nemertini*, should acknowledge that the axis cylinder is homogeneous, and yet conclude that it is solid and non-fluid in consistency. He reached this result as to its density simply from observations with polarized light; for the axis cylinder would be capable of isolation, provided that its sheath remained intact, even if it were fluid in consistency. Not, I think, that the best reason for the correctness of the assumption, that the substance of the axis cylinder has a fluid or viscid, and not a dense, consistency, is to be given by the fact that this substance is in every way identical with the hyaloplasm of the cell; and all observations tend to prove that the hyaloplasm of the cell cannot be of solid (dense) consistency, but is fluid, or at least viscid.

The structure of the nemertean nerve tubule, as just described, may be seen after preparation with corrosive sublimate, but is rendered much more distinct after fixation in Flemming's or Hermann's fluids. The fluid of Hermann especially renders the course and structure of the nerve tubules very plain, and for this purpose cannot be too highly praised. Thus, after fixation of half an hour in this fluid, the spongioplasmic sheaths of the tubules and all the neuroglia fibres (or "fibrils," as one will) of the fibrous core of the brain and lateral chords become stained a deep bronze-brown, while the hyaloplasmic axis cylinders remain unstained, and are easily traceable as fine, colorless lines. And this result is reached after sectioning in paraffine and mounting in Canada balsam, — a course of procedure much deprecated by Apáthy ('91). Hermann's fluid, accordingly, produces just the very opposite effect of the impregnation method of Golgi: by the former only the axis cylinders become stained, while by the silver method it is especially these parts which become blackened. But Hermann's fluid is always precise in its effect, while, as Friedländer ('89) has shown, the Golgi method is far from reliable, since frequently neuroglia, as well as the strictly nervous elements, become stained by it.

The nerve tubule is best studied in its proximal portion (Figs. 3–14), since it has the greatest diameter at this part; and further, owing to its somewhat tortuous course within the fibrous core, it is only this portion which may be traced to the ganglion cell (cf. Fig. 3). In a small number of cases I have found spongioplasmic strands or fibres prolonged from the cytoplasm of the cell for a short distance into the hyaloplasm of the axis cylinder (Figs. 4, 5, 7, 11, 13, *F.*). These strands of spongioplasm are found only in the most proximal portion of the axis cylinder, and are not continued distally. The observation of such fibres as these might have led Nansen ('87) to the conclusion that the axis cylinder represents a bundle of "primitive nerve tubes," and that these fibres correspond to their supposed sheaths. For my part, however, I consider that the nemertean axis-cylinder process represents a single nerve tubule, and not a bundle of parallel "primitive nerve tubes." And the spongioplasmic strands or fibres which I have described in the axis cylinder, (1) being found in but a small proportion of the large number of axis cylinders examined, (2) being even then present only in their most proximal portions, and (3) varying both in number and size (Figs. 4, 5, 7, 11, 13), would lead to the justifiable conclusion that they do not represent sectioned sheaths of "primitive nerve tubes," but are merely spongioplasmic prolongations of the cytoplasm of the ganglion cell, which penetrate but a short distance into the hyaloplasm of the axis cylinder.

On cross section, studied with high powers of the microscope, the nerve tubule appears as a minute, circular, unstaining disc, bounded by a fine, staining line (Fig. 39, *Ax. Cl.*, also Figs. 36, 37).

As noted above, the nerve tubule has the greatest diameter proximally, and distally gradually decreases in size (Figs. 3, 4). There are apparently no dichotomic divisions by which two branches of equal diameter are produced. But the diminution in calibre is due, partly at least, to the giving off of collaterals from the nerve tubule, which always have a smaller diameter than that of the nerve tubule at the point of division (Fig. 39, *Coll.*). I have never been able to trace these collaterals for

more than a very short distance, nor have I found them branched; my observations corroborate those of Bürger ('91b) on methylene blue preparations, who discovered the collaterals (his "ungleichwerthige Nebenfortsätze"), and found them always unbranched. After the nerve tubule has given off a number of collaterals, it is greatly decreased in diameter, becoming almost invisible. In this distal portion of its extent, the finer structure can necessarily be no longer determined; but there is no adequate reason for supposing that the structure of the nerve tubule here differs from that in its proximal portion, as maintained by Bürger ('91b). The nerve tubule, after entering the fibrous core, certainly does not divide into a brush of fine branches, as figured by Rohde ('90a) for polychaetes.

It remains to state that even after the use of fluids containing OsO_4 I have been unable to find any evidences of a myelin sheath, such as has been described by Retzius ('89b) and Friedländer ('89) for other invertebrates. And as little as H. Schultze ('79) and Apáthy ('91), have I found varicosities of the axis cylinder.

In *Cerebratulus lacteus* I could not make as thorough a study of the nerve tubules as in the preceding species, since I had but one specimen, and that fixed with alcoholic sublimate. But it may be stated that these preparations of *Cerebratulus* showed the same structure, as those of *Lineus* fixed by the same method. The spongioplasmic, peripheral layer of the ganglion cell is produced to form the sheath of the nerve tubule, and its hyaloplasm is in direct connection with that of the axis cylinder (Figs. 20, 21, 23, 29, *III*). As in *Lineus*, spongioplasmic strands sometimes penetrate from the cytoplasm of the cell into the proximal portion of the axis cylinder (Fig. 23); but though found in a comparatively larger number of cells than in that genus, they are nevertheless restricted to relatively few cells. Such strands are of varying thickness, and have a greater diameter proximally than distally; further, they vary in number from one to about six, and, though more or less parallel in position, are not placed at equal distances apart. Accordingly, there is apparently no regular distribution of the spongio- and hyaloplasmic substances in the proximal portion of such an

axis cylinder, *e.g.*, no alternating, concentric, or linear zones of these substances. In short, the nerve tubule in the proximal and distal portions of its extent consists, as in *Lineus*, of a thin spongioplasmic sheath and an enclosed, homogeneous, unstaining, hyaloplasmic axis cylinder; only in a small number of cases are spongioplasmic fibres or strands, which vary in size and number, continued from the framework of the cell for a short distance into the hyaloplasm of the proximal portion of the axis cylinder.

The nerve tubules of cells I are so minute in diameter that their structure can only be studied in their wider, proximal portion (Figs. 1, 17); as far as I could determine, they have the same formation as those of III. In Fig. 37, representing a cross section of the fibrous core of the dorsal brain lobe, the unstained axis cylinders are probably of both cells I and III (these two types of cells forming the ganglion-cell layer of the dorsal lobe). The structure of the nerve tubules of cells II (Figs. 2, 18) may be more easily studied than those of I, and have exactly the same structure as the nerve tubules of III, from which they differ only in size.

It will be well to review briefly Bürger's representations of the structure of the nerve tubules of cells III before describing those of IV.

Bürger's observations ('90b, '91b, '95) differ quite essentially from mine, though his direct observations less so than his generalizations. For the terms "spongioplasm" and "hyaloplasm" used here, he adopts Flemming's expressions, "mitom" and "paramitom." The following quotations will show his views on the ganglion-cell processes ('95): "Die Fortsätze der Ganglienzellen, welche den Stammfortsätzen von Retzius ['90] entsprechen, zeigen mit Ausnahme derjenigen der Neurochordzellen perlschnurartige Verdickungen" (p. 332). . . . "Die Nervenfaser der Centralsubstanz d. i. der in ihr enthaltene Abschnitt des Stammfortsatzes stellt nämlich nicht einfach den in den Seitenstamm der Nemertine verlängerten Fortsatz der Ganglienzelle dar. Denn der Fortsatz einer Ganglienzelle verjüngt sich in der Centralsubstanz oft bis in eine recht feine Spitze und tritt mit dieser an die Nervenfaser heran, die häufig

diesen Fortsatz überhaupt, jedenfalls aber sein verjüngtes Ende sehr an Dicke übertrifft" (p. 334). The "Nervenfibrillen" of all cells but IV possess "Nebenfortsätze": "Unsere Nebenfortsätze entspringen von den Verdickungen [varicosities] der Fibrillen. . . . Ich halte den Stammfortsatz der Ganglienzelle, obwohl ich an ihm zwei Abschnitte ["Ganglienzellfortsatz," "Fortsatzfibrille"] unterschied, keineswegs für ein zusammengesetztes Gebilde im Sinne von Dohrn ['91, the view has since been relinquished by Dohrn] und Apáthy" (p. 335).

Now the Göttingener investigator distinguishes on the "nerve fibre": (1) the true prolongation of the ganglion cell, and distally (2) the "nerve fibril," which he claims is sharply bounded off from the former, though both should stand in the same genetic relationship to the cell! He found, with the *intra vitam* methylene blue staining, that the distal "Fibrille" stains more intensely than the proximal "Ganglienzellfortsatz"; he gives no structural details of its formation, but his term "Fibrille" leads us to suppose that he conceived of it as a dense fibril with a structure different from that of the proximal process. His descriptions would give the idea, further, that there is an abrupt local as well as structural demarcation between the two, *i.e.*, that they do not merge gradually into each other. Already in a former paper Bürger ('90b) had distinguished ganglion-cell process and nerve fibril on the study of sections fixed with chromic acid and with alcoholic sublimate (?); of the structure of the ganglion-cell process, he states (p. 112): "Bei III und IV ist das Paramitom vorherrschend. Es ist in mehreren Säulen im Fortsatze gelagert, jede Säule wird von einer Mitomscheide umgeben, das gesammte Bündel umfasst eine besonders körnige Mitomschicht, wie sie als Rindenschicht des Zelleibes charakterisirt wurde." This representation of the structure of the proximal portion of the axis cylinder, namely, as a nerve tube composed of a bundle of "primitive nerve tubes," is in close accord with the descriptions of Nansen ('87) for other forms; and Bürger's Fig. 56, Taf. IV, bears marked resemblance to certain of Nansen's figures. Finally, of the nerve fibril, he states ('90b, p. 118): "Als Formelement der centralen Fasermasse habe ich eine

feinste, kaum messbare Fibrille verfolgen können, die in den Ganglien einen verschiedenen Verlauf besitzt, in den Kommissuren von einer Gehirnhälfte zur anderen ihren Weg nimmt und in den Seitenstämmen entschieden längsgerichtet ist."

Now I have found that the nerve tubule, as far as it can be followed, consists of a thin spongioplasmic sheath and an enclosed, homogeneous, hyaloplasmic axis cylinder (Figs. 3, 36–39). The axis cylinder is not a bundle of "primitive nerve tubes," as held by Nansen, and as Bürger's ('90b) Fig. 56 would show. The assumption of the existence of the "primitive nerve tubes" has probably had its origin in that those spongioplasmic strands, which I have shown to be sometimes prolonged for a short distance into the axis cylinder, had been regarded by Nansen and his followers as the sheaths of such primitive tubes; that such a structure is not present in the nemertean axis cylinder I have already shown. My results would show that the axis-cylinder process in its whole extent represents a single nerve tubule; and that a structural distinction into a proximal "ganglion-cell process" and a distal "nerve fibril" is not present. A "kaum messbare Fibrille," in the sense in which Bürger uses the term, namely, as a dense, deeply staining, non-tubular fibril, I have never found. And in fact, I have found only a number of parallel nerve tubules at the roots of the spinal nerves and in the first ventral commissure of the brain,—points where Bürger stated his "nerve fibrils" to be most easily demonstrable. Therefore, Bürger, in those studies ('90b) of his based upon stained sections, had either: (1) supposed the true hyaloplasmic axis cylinder to be simply an unstaining space between two "fibrils," and then had mistaken the sheaths of the axis cylinders for "fibrils"; or (2) what, however, seems to be a less probable explanation, had confused neuroglia fibrils with "nerve fibrils." But we must conclude that in the fibrous core he had not seen the true axis cylinders. This critique cannot be considered unjust, since Bürger had neglected to employ the two methods best qualified to demonstrate the axis cylinders in the fibrous core, namely, Flemming's fluid and, better still, the fluid of Hermann.

But does the nerve tubule consist of two separate parts, — a proximal ganglion-cell process and a distal "Nervenfibrille"? Bürger's ('91b) figures drawn from preparations stained in life with methylene blue certainly seem to support such a view. But his descriptions do not mention any difference of structure between the two parts, but simply a difference in dimension and a slight distinction in staining power; and my own observations have taught me that there is no structural distinction between the proximal and distal portions, but only a gradual difference of diameter. Thus, if the nerve tubule be divisible into a proximal ganglion-cell process and into a distal portion, this distinction will probably be found, not to be one based upon differences of structure in the nerve tubule itself, but either upon differences of dimension or of differences in the structure of its connective-tissue envelope (sheath of Schwann). And with Apáthy ('91), I think the supposed varicosities of the nerve tubule might also be referred to the sheath of Schwann.

B. *Cell IV.*

The nerve tubules of the cells IV are the largest in the nemerteans, and deserve the term "neurochords" applied by their discoverer, Bürger ('90b), with reference to their similarity to the neurochords (Eisig, '87; "Leydig'sche Fasern," Friedländer, 89) of the annelids and crustaceans.

This colossal nerve tubule (Figs. 27–29, 32, *Ax. Cl.*) is composed of a thin spongioplasmic sheath, which is a continuation of the peripheral alveolar layer of the cell; and of an enclosed, unstaining, homogeneous, hyaloplasmic axis cylinder, which is in direct communication with the hyaloplasm of the distal portion of the cell. The neurochord nerve tubule differs from the tubules of the other ganglion cells mainly in its superior size. I have never found any evidences of a finer structure in the hyaloplasmic axis cylinder, but always found it to be homogeneous; my results being in accord with those of Friedländer ('89) on annelids and crustaceans, and with those of Apáthy ('91) on *Hirudinea*. But it is to be noted that on studying, for example, a longitudinal section of such a neurochord by elevating or

lowering the tube of the microscope, *i.e.*, with a focus a little too high or too low, the enveloping sheath of Schwann is brought into view, presenting the illusory appearance of a stained structure lying in the axis of the neurochord. It is possible that Bürger ('90b) had experienced such an optical illusion when he described an irregular, staining structure lying in the axis of the nerve tubule in *C. marginatus;* otherwise it must have been an artifact, as I found no positive evidences of such a structure in *C. lacteus*. On cross section (Figs. 30, 40, *Ax. Cl. IV*), the neurochord is found to consist of an unstaining, homogeneous disc (the hyaloplasmic axis cylinder); this is immediately bounded by a very fine spongioplasmic layer, and outside of the latter is the thicker, granular sheath of Schwann, which stains with eosin. I have been unable to find traces of a myelin sheath; but since the individual examined had been fixed in 50% alcoholic solution of sublimate, alcohol having the property of dissolving myelin, the nemertean neurochord must be studied after the action of a fixative containing OsO_4 (this acid blackening myelin) before it can be determined whether such a sheath is present or absent. And this point is worth investigation with regard to the possible homologies of these neurochords with those of the annelids, crustaceans, *Amphioxus*, and even *Myxine*.[1]

The neurochord of cell IV divides dichotomously, though the points at which it divides are situated at considerable distances apart. Fig. 42 *a*, *b* shows such dichotomic divisions on longitudinal sections. It is the rule that each of the branches has one-half the diameter of the neurochord before the point of division, so that it is a strictly equal dichotomy. In an exceptional case (Fig. 42 *c*) were two branches given off at the same point from the one side of the neurochord, though the diameter of the latter behind this point was not diminished; this should, perhaps, be regarded as a pathological case, and these two branches would not correspond to collaterals, since Bürger ('91b)

[1] H. Schultze ('79) first discovered myelin in the nerves of invertebrates within the substance of the axis cylinder (between his "primitive fibrils") in *Unio* and *Anodonta*. Myelin sheaths of the nerve fibres of vertebrates have been described by Leydig ('85), Retzius ('89b), Friedländer ('89), for annelids and crustaceans, and by Apáthy ('91) for *Hirudinea*.

has found the absence of collaterals characteristic for the neurochords. Leydig ('85) has figured a dichotomic division of the neurochord in *Dytiscus*. Another peculiarity of these neurochords I find in their constrictions, which may be compared to the "anneaux constricteurs" ("Ranvier'sche Einschnürungen") of the vertebrate axis cylinder. To wit, just anterior to a point of division, such a constriction of the axis cylinder may always be found (Fig. 42 *a*, *b*) ; and in one case (Fig. 42 *d*) I found a constriction far removed from any point of dichotomy. Such segmental constrictions of the neurochords have been observed by Friedländer ('89) in the polychaete *Mastobranchus;* but in this annelid the constrictions are of greater extent than in *Cerebratulus*. It will be recalled that three pairs of cells IV occur in the ventral lobes of the brain, and none at all in the lateral chords in the oesophageal region ; each lateral chord, therefore, derives three neurochords from the respective side of the brain. But these most anterior neurochords do not pass undivided through the lateral chord in its oesophageal region, but divide here dichotomously ; so that in studying a series of transverse sections from this region, they are found to increase in number and decrease in size posteriorly. Since, however, on a cross section of the lateral chord in the posterior portion of this region it is not infrequent to find a neurochord fully as large in diameter as one in the more anterior portion, we must suppose, either (1) that a neurochord or one of its branches may vary in diameter in different portions of its course, or (2) that a number of neurochords may fuse together, posteriorly, to produce a larger, compound neurochord (or neurochord "bundle"). Though the first alternate would appear to be the more probable, I was unable to determine this question, even on longitudinal sections.

Bürger first ('90b) maintained that the neurochords of *Cerebratulus* and *Langia* divide dichotomously, after observing their numerical increase on successive cross sections. Later ('91b) he abandoned this view, and finding on *intra vitam* methylene blue preparations no branching of the neurochords, concluded that the latter pass to the posterior end of the lateral chords without division. My observations, which are epitomized in

Fig. 42 *a–e*, corroborate his former view, and show that the neurochords possess equal dichotomic divisions.

Though I have followed Bürger ('90b) in applying the terms "neurochord cells" and "neurochords" to nemerteans, — terms introduced by Eisig ('87) for the colossal ganglion cells and their fibres in annelids (which structures had been recognized first by Leydig, '64, as nerve tubules), — yet I would not intend to express, by the use of similar terms, homologies between these structures in different animal groups. Friedländer ('89) maintained such a homology in different animal groups, or at least declared the neurochords of different groups to be "fundamentally the same" structures (p. 258). But what is meant by the expression "fundamentally the same," if not homology? But though the neurochords may present similar structural relations in the different animal groups (*Nemertini*, *Annelida*, *Arthropoda*, *Leptocardia*), their homology is not thereby proved; for there is as much probability for the assumption that they have been developed independently by these various forms. And notwithstanding the large number of investigations already made upon neurochords,[1] it is still necessary to subject them to renewed study, with the aid of the newer histological technique, and to treat them from the comparative standpoint.

IV. Neuroglia.

As Bürger ('90b) has demonstrated, the brain lobes and lateral chords are compounded of the following layers: (1) the outer neurilemma (Fig. 25, *o. Neur.*), a connective-tissue capsule enveloping (2) the ganglion-cell layer, and (3) the inner neurilemma (*i. Neur.*), which separates the latter layer from (4) the central fibrous core (composed of "dotted substance"). Bürger had also described the connective-tissue elements of the ganglion-cell layer, and termed it "intracapsuläres Bindegewebe" or "Hüllgewebe"; he discovered, also, a layer of cells of this tissue situated between the inner neurilemma and the

[1] A nearly complete bibliographical list of papers dealing with neurochords has been given by Friedländer ('89).

fibrous core. In a paper ('97) on the cytology and comparative distribution of the connective-tissue elements in the nemerteans (*Carinella*, *Cerebratulus*, *Lineus*, *Amphiporus*, *Tetrastemma*, *Stichostemma*), I have given detailed descriptions of the cellular elements forming the neurilemmatic capsules, and the "Hüllgewebe" (this latter I termed "neuroglia"); for fuller structural details of these elements, I must refer to the paper in question.

Now I agree with Bürger's conclusion ('90b, p. 137): "Das Pigment führende Hüllgewebe der Ganglienzellbündel . . . und der faserig-zellige Mantel des Faserstammes . . . sind ursprünglich ein und dieselben Gewebsgebilde, örtlich von einander getrennt und differenzirt." But he adds (p. 138): "Sie sind vielleicht als mit dem Neurilemma in Gemeinschaft entstanden zu denken, jetzt aber so sehr von den Bildungen desselben verschieden, dass sie für sich zu betrachten sind. So sind sie auch mit der Glia (Hatschek's) unvereinlich, da sie ihrem vornehmsten Kriterium Scheiden- oder Röhrenbildnerinnen niemals entsprechen." I have shown in my previous paper (*l. c.*), however, that the elements of the neuroglia (Bürger's "Hüllgewebe") and those of the neurilemmatic sheaths must be considered as distinct for the following reasons: (1) because the tissue of the neurilemma is identical with the connective tissue forming the basal membranes of all epithelia, with that enveloping the muscle bundles, etc., and consists of branched cells lying within a dense intercellular substance, while the neuroglia is restricted to the nervous system, and its cells are not imbedded in an ectoplastic, intercellular substance; and (2) because no intergradations between these two tissues occur, not even in that primitive form *Carinella*. Further, as shall be described, the branching fibres of the multipolar neuroglia cells not only produce a loose network around the ganglion cells (III, IV), but also form the outer connective-tissue sheaths of their nerve tubules. Thus Bürger's "Hüllgewebe" is a typical neuroglia tissue, and can stand in no histogenetical relationship to the tissue of the neurilemmatic sheaths. It is more probable, indeed, in the light of our knowledge of the ependyma (embryonal neuroglia) in vertebrate histogenesis, that the neuroglia of the nemerteans stands in closer genetical relation

to the ganglion cells themselves than to the cells of the neurilemma (cf. Rohde, '90b).

I have ('97) found that the neuroglia of the central nervous system occurs in two or three modifications, according to structural differences: thus (1) the "outer neuroglia," which is situated between outer and inner neurilemma, and (2) the "inner neuroglia," in and around the fibrous core, may be distinguished in all nemertean genera; while in a few genera the outer neuroglia may be subdivided into (a) the outer neuroglia of the brain lobes, characterized by the presence of pigment, and (b) the outer neuroglia of the lateral nerve chords, in which pigment is absent.

The membraneless, multipolar cells (Figs. 35 *a–c*, 40, *Ngl. C.*) of the outer neuroglia, which are situated between the outer and inner neurilemma of the brain and lateral chords, possess long, fine, branching fibres which produce loose fibrous sheaths around each of the ganglion cells of the types III and IV. In the Figs. 3–16, 19, 21, 23, 27–29, 32 the fibres alone of the sheaths (*Ngl.*) are reproduced, the cells from which they arise, and which usually are more peripherally situated, having been figured in my preceding paper (*l. c.*). Bürger ('90b) first described these fibrous sheaths of the ganglion cells; but he had overlooked the more important fact that they are continued along the nerve tubules also, producing an outer connective-tissue sheath of the latter, which is fully comparable to the sheath of Schwann ("Schwann'sche Scheide") of, *e.g.*, the vertebrate axis cylinder, and for which the same term may be applied. The number of fibres composing the sheath of the ganglion cell is greatest and they are most loosely arranged around the enlarged proximal portion of the cell (Figs. 3–8, 10–13, 19, 21, 23, 27–29, *Ngl.*). Towards the distal pole of the cell — the point of origin of the nerve tubule — the number of enveloping neuroglia fibres diminishes, and they commence to place themselves much closer to this portion of the cell. Finally, on each side of the proximal portion of a nerve tubule, as seen on a thin longitudinal section, one neuroglia fibre may be seen, and can be traced to one of the fibres forming the fibrous sheath of the ganglion cell. Thus the nerve tubule is enveloped by a

sheath of Schwann (*S. Sh.*), the separate longitudinally directed fibres of which are derived from the fibrous sheath of the cell itself. As far as I could determine, the sheath of Schwann consists of a single layer of longitudinal neuroglia fibres, the fibres being parallel to, but not in contact with, one another, *i.e.*, they apparently do not produce a continuous membrane. I could not determine along how great an extent of the nerve tubule this sheath continues, since its fibres decrease in size distally. Certainly, however, in its proximal portion the following components of the nerve tubule may be distinguished: (1) the axial, hyaloplasmic axis cylinder, (2) the spongioplasmic sheath of the latter, and (3) on the outside of this sheath, the sheath of Schwann. On a distal portion of the nerve tubule the axis cylinder is found to be bounded by a very fine, scarcely visible envelope (Fig. 40); the latter is so minute that one cannot determine whether it corresponds to the spongioplasmic and the neuroglia sheath, or whether to merely one of the latter.

On longitudinal sections of the neurochord nerve tubules (Fig. 42 *a–e*) the separate fibres of the sheath of Schwann are mostly found to be bisected; this fact might be explained on the ground that they describe a spiral course around the nerve tubule.

As just described, the sheath of Schwann is formed by a continuation along the nerve tubule of certain of those outer neuroglia fibres which produce the loose sheath around the proximal portion of the ganglion cell. But whether fibres from the cells of the inner neuroglia also take part in the construction of the sheath of Schwann, I have been unable to decide; this might appear probable in those cases where the distal pole of the ganglion cell lies close to the layer of the inner neuroglia cells. Further, I cannot state positively that an additional sheath, derived from the inner neurilemma, is added to the nerve tubule; though in support of there being such a sheath around at least the proximal portion of the tubule, I may refer to the left-hand cell portrayed in Fig. 29, where a thin plate of the inner neurilemma is split off from the latter, and accompanies the proximal portion of the nerve tubule for a short distance. This was the only case in which I found evidences of such a neurilemmatic sheath.

V. DOTTED SUBSTANCE OF THE FIBROUS CORE.

The fibrous core (Figs. 25, 30, 35 *a–c*, 40, *F. C.*) of the brain and lateral chords is composed of the so-called "dotted substance" ("Punktsubstanz" of Leydig), and is divided from the outer ganglion-cell layer by the inner neurilemma (*i. Neur.*). The only cells occurring within the fibrous core are the branched cells (*Ngl. C.*) of the inner neuroglia tissue, and these are situated mostly peripherally, just beneath the inner neurilemma, though a small number penetrate also into the dotted substance.

The fibrous core is composed of: (1) fibres of the cells of the inner neuroglia, which compose its greater part, (2) nerve tubules and their sheaths, (3) irregular spaces containing body fluid. Apparently no fibres or strands of the inner neurilemma penetrate for any considerable distance into it. It cannot be too strongly emphasized, that in order to arrive at a clear understanding of this much-discussed portion of the central nervous system, comparisons must be made between preparations fixed in different fluids (especially Hermann's fluid and sublimate solutions), and between those differentiated by different stains. Since the structure of the fibrous core differs somewhat in *Lineus* and *Cerebratulus*, it may be described separately for these two genera.

A. *Lineus.*

In this genus there is a proportionately larger number of inner neuroglia cells around the fibrous core than in *Cerebratulus*, and their fibres are also coarser and hence more easily followed. These fibres, as well as the cell bodies, are of a dense, granular structure, and stain intensely with eosin; and though they branch excessively, I have never found anastomoses between the fibres of adjacent cells.[1] The fibres of the peripheral inner neuroglia cells radiate into the fibrous core, producing the fibrillar, staining substance of the latter. A single neuroglia fibre with its branches may often be traced for a considerable

[1] If it were necessary to be precise in our terminology, the term *fibre* could be restricted to the branch of the cell, and *fibril* employed for secondary branches of these fibres; but these distinctions are of doubtful value, and need not be employed here.

distance in the fibrous core, and is always to be distinguished from the really nervous elements by (1) its granular, refractive appearance, (2) its staining power with eosin, (3) its irregular branches. On cross section, after fixation in sublimate, such a fibre appears as a refractive, staining granule or group of smaller granules (depending upon its size).

The nervous elements in the fibrous core, or dotted substance, are the nerve tubules, and their course and grouping is to be best studied on sections of material fixed with Hermann's fluid (in Figs. 36–38 I have tried to reproduce the appearance of such preparations, though the ground color should be more of a bronze than green). The axis cylinder of the nerve tubule never stains (except with methylene blue, and probably with gold chloride: cf. Apáthy, '91), is circular on cross section, and its fine, staining envelope is the spongioplasmic sheath. After fixation in Hermann's fluid for half an hour, when studied with high magnifying powers (Fig. 39), the fibrous core is seen to consist of a deeply stained, very finely granular matrix (of neuroglia fibres), throughout which the unstaining axis cylinders lie; with a lower power of magnification (Figs. 36–38) the staining matrix appears homogeneous, deeply staining, and the axis cylinders as fine, colorless lines, or dots on cross section. Sublimate produces a more coarsely granular appearance of the fibrous neuroglia matrix than does Hermann's fluid, probably owing to the faculty of the former reagent to produce coagulation.

In the fibrous core of the dorsal (Fig. 37) and ventral lobes of the brain and in the dorso-ventral commissure the unstaining axis cylinders are more or less regularly distributed, and are not united into special bundles. In the dorsal commissure of the brain (commissure of the dorsal lobes) and in the ventral commissures (Fig. 38), they all take a parallel course, but here also are not grouped into bundles.

There is in the lateral chord, however, a more specialized arrangement of the nervous elements (Fig. 36). Here, namely, a short distance behind the ventral lobe of the brain, about the region of a frontal plane passing through the posterior end of the cephalic sense organ, a large tube arises (Fig. 36, *N. T.*) which is situated a little lateral from the center of the fibrous

core and courses towards the posterior end of the chord, where it becomes gradually indistinguishable, owing to decrease in diameter. On cross section it is more or less circular or oval in outline, and is bounded by a fine sheath; its contents do not stain and are homogeneous, except that occasionally vestiges of a very fine, reticular structure may be found within it. On account of its large size and general appearance, especially on longitudinal sections, it bears a close resemblance to a neurochord; but neurochord cells are absent in *Lineus*. This structure certainly can represent nothing else than a bundle of nerve tubules, and accordingly may be termed a *nerve tube;* the fine reticulation which its contents sometimes show would correspond to the sheaths of the individual nerve tubules composing it. It is the "Faserstrang" found by Bürger ('90b, '91b), but it is not composed of a bundle of "nerve fibrils." The fine membrane surrounding the nerve tube is so minute that I cannot determine whether it is a neuroglia product *sui generis*, or whether it is formed by a coalescence of the sheaths of the outermost layer of enclosed nerve tubules. This tube is not continued, anteriorly, into the ventral lobe of the brain, nor into the first commissure of the latter. Further, it does not persist as a single, unbranched tube through the whole length of the lateral chord. For on following a series of cross sections, the first section may show a single tube, the second two tubes (each half the diameter of that on the foregoing section), further sections may show three or four tubes, and a consequent section the disc of but one tube again (Fig. 33 *a–d* shows the branching of the nerve tube as seen on four consecutive cross sections). That the tube branches, apparently dichotomously, can be observed on longitudinal sections also. Thus the nerve tubules in the nerve tube do not pass singly out of the latter into the peripheral nervous system, but smaller tubes (each containing a number of nerve tubules) branch off from the main tube. It is possible that towards the posterior end of the lateral chord, where the diameter of the main nerve tube becomes much reduced, the nerve tubules pass out of it singly; but I do not attempt to decide this point, which has no direct bearing upon the questions at issue.

A large number, though by no means all and perhaps not even one-half, of the nerve tubules of the lateral chord are enclosed in this nerve tube. For a glance at Fig. 36 demonstrates that a considerable number of cross-sectioned axis cylinders are distributed pretty evenly throughout the fibrous core, without being grouped together as nerve tubes. In fact, there appear to be nearly as many nerve tubules scattered throughout the fibrous core of the lateral chord, and not enclosed in its central nerve tube, as we find in the fibrous core of the dorsal and ventral brain lobes, where no nerve tubes occur (cf. Fig. 36 of a lateral chord with Fig. 37 of the dorsal brain lobe).

For each nerve tubule of cell III there is usually a special opening in the inner neurilemma, while, as already noted, for each radial cluster of cells II (Fig. 25) there is but a single opening through which all their nerve tubules penetrate to the fibrous core. Fig. 36 shows that the bundle of nerve tubules from such a cluster of cells II penetrates nearly to the center of the fibrous core, where the individual nerve tubules then diverge and bend towards the posterior end of the chord. Fig. 37, representing a transverse section of the core of the dorsal brain lobe, exhibits the same phenomenon in regard to clusters of cells I. Probably new accessions of nerve tubules are being continuously added to the central nerve tube of the lateral chord from such metameric bundles of nerve tubules of the radial cell clusters of II.

Attention may be drawn to Fig. 41, from a preparation hardened in sublimate, afterwards stained with methylene blue followed by brasilin, of *Tetrastemma vermiculum*. In the longitudinally sectioned fibrous core (*F. C.*) of the lateral chord, I find a rod-like structure which had been stained a deep green. This body probably represents just such a nerve tube as I have described for *Lineus*.

I must not be misconstrued into supposing that all the unstaining portions of the dotted substance represent axis cylinders. On the contrary, I have found irregular, even more or less tubular, unstaining spaces in the fibrous core which can represent nothing more nor less than spaces filled with body fluid, just as the unstaining spaces enclosed by the muscle

bundles of the body wall.[1] Thus in those frequent points in the brain lobes and lateral chords where "strings" of the inner neuroglia cells penetrate into the fibrous core, these cellular ingrowths do not form compact masses, but their individual cells are separated by irregular, unstaining spaces, which branch out into the substance of the fibrous core. Often such irregular, branching cavities are present beneath those inner neuroglia cells which occur on the periphery of the fibrous core (Fig. 37, *Sp.*). The clefts or spaces produced by these accumulations of body fluid may readily be distinguished from cross-sectioned axis cylinders by their superior size, irregular form, and absence of a limiting sheath.

The results of Bürger's ('90b, '91b, '95) observations on the finer structure of the fibrous core differ quite essentially from my own. For he supposed its nervous elements to be dense, staining "nerve fibrils," a view which my investigations would show to be erroneous, and which has been sufficiently criticised above. He consequently considered that the minute, unstaining spaces of the fibrous core did not represent axis cylinders, but clefts filled with body fluid, showing that he had overlooked the true nerve tubules. Again, the nerve tube (*mihi*) of the lateral chord, which he discovered and termed the "Faserstrang," he stated to be composed of a bundle of deeply staining "nerve fibrils," whereas I was able only on a few preparations to find evidences of a structure in it, and then in the form of a faintly staining reticulation (never "nerve fibrils"), which probably represents the sheaths of enclosed nerve tubules. Bürger may have employed a fixing reagent which had caused such an excessive coagulation of the contents of the nerve tube as to have given the appearance of fibrils; but, as already stated, I have never found any evidence whatever of the

[1] As a rule, the body fluid of the nemerteans can be considered to be a thin, homogeneous, unstaining fluid, which fills the very numerous, usually minute, clefts in the different tissues; but I have shown ('97) that that portion of it enclosed in the bundles of the longitudinal musculature, where it is found in great quantity, becomes to a certain degree coagulated by corrosive sublimate, and then presents a finely granular appearance. Before this observation was made, no one had actually seen the body fluid, although previous investigators had assumed its existence.

existence of such fibrils, though I have worked with a larger number of preserving and staining reagents. He described the "Faserstrang" of the lateral chord in *Cerebratulus marginatus* as continued directly into the fibrous core of the ventral brain lobe; in *Lineus* I have found no such anterior continuation. Lastly, Bürger had not noticed the branching of the nerve tube (his "Faserstrang").

Rohde, who previously maintained ('90a, '90b) that the fibrillar structures of the dotted substance were the true nervous elements, has later ('92) abandoned this view and, following Leydig ('64), assumed the hyaloplasm to represent the nervous elements.

B. *Cerebratulus.*

The dotted substance of *C. lacteus* was studied on sections fixed with alcoholic solution of sublimate; and since I had no material hardened in Hermann's fluid I could not determine the course of the smaller nerve tubules, and only to a certain extent that of the neurochords.

The structure of the fibrous core of the lateral chords is illustrated by the Figs. 30, 35 *a–c*, 40; the same structure is found in the brain lobes, except that here the neurochords are absent. As may be seen in Fig. 40, which represents a lateral segment of a cross section out of about the middle point of the fibrous core of the lateral chord, the dotted substance consists, as in *Lineus*, of: (1) the staining fibres of the inner neuroglia cells (*Ngl. C.*); (2) axis cylinders (*Ax. Cl.*) and their sheaths; (3) irregular, unstaining spaces between the latter which, in reference to what we have learned in *Lineus*, are probably filled with body fluid. But the fibrous core of the lateral nerve chords in *Cerebratulus* differs especially from that of *Lineus* in the presence of the colossal nerve tubules (neurochords of cells IV, *Ax.Cl. IV*, Figs. 30, 35 *b*, 40). I have found these neurochords to be pretty equally distributed throughout the fibrous core, though they are more numerous centrally and laterally than medially (Fig. 30). In the oesophageal region I traced for a considerable distance a neurochord, which was situated at the latero-ventral corner of the chord. The fibrous core pre-

sents a somewhat different structure in different portions of the lateral chord: thus anteriorly (Fig. 35 *a*) the finer nerve tubules preponderate in number, and but few neurochords occur, while more posteriorly (Fig. 35 *b*, *c*) the neurochords increase in number, which is due to their dichotomic divisions as well as to the accession of new neurochords from the cells IV placed along the chord, these cells becoming more numerous posteriorly.

Thus I have found no nearly central bundle of neurochords, such as Bürger (91b) has described, but rather individual neurochords of various diameters as well as the smaller nerve tubules (of cells II and III) throughout the whole diameter of the lateral chord. Although I did not observe a nerve tube in the lateral chord, such as has been described for *Lineus*, I would not thereby maintain the absence of such a structure in *C. lacteus*, since it might here easily be confounded with a neurochord.

VI. Brain Commissures, Oesophageal Nerves.

The large ventral commissure of the brain which had been distinguished by the pioneers of nemertean anatomy, Delle Chiaje (1823) and Quatrefages (1846), has been supposed by all succeeding investigators to be the only ventral commissure. This large commissure may be termed, however, the *first ventral commissure* to distinguish it from two posterior commissures which are described here for the first time, and which may be termed the *second* and *third ventral commissures* respectively. In *Cerebratulus lacteus* the second ventral commissure arises from the fibrous core of each ventral lobe, just ventral to the oesophageal nerves, and one section behind the first pair of neurochord cells of the brain. It has scarcely one-fifth the diameter of the oesophageal nerve at this point, and, though it apparently has no special neurilemmatic envelope, is separated from this nerve by the neurilemmatic sheath of the latter. The nerve tubules of which this commissure is composed (probably those of cells II and III) are derived from the fibrous core of either ventral lobe. In *Lineus gesserensis* (Fig. 43) this second ventral commissure (*Comm. 2*) has the same position and struc-

ture as in *Cerebratulus;* but in *L. sp.*, in addition to this second commissure, there is also a third ventral commissure (absent in the two preceding species) which is placed two sections (about 7μ) behind the second.

The paired oesophageal nerves of *Cerebratulus lacteus* ("vagus nerves," according to Hubrecht, who sought to homologize them with the vertebrate vagi), I find to have three commissures, as Bürger ('90b) has described for *C. marginatus;* while Coe ('95a), who also studied *C. lacteus*, saw only the third (largest) commissure. Each oesophageal nerve has its origin on the median side of the fibrous core of the ventral brain lobe, a short distance behind the first ventral commissure of the latter; the nerve is therefore, in point of origin, a posterior continuation of part of the fibrous core of the ventral lobe. In this core the nerve has its own sheath, which is a derivative of the inner neurilemma of the former. About five sections behind the second ventral commissure of the brain lies the *first commissure* of the oesophageal nerves, the latter commissure being but little thicker than the former. From this point on, these nerves are situated outside of the fibrous core of the brain lobe. Three sections behind the first lies the *second commissure* of the oesophageal nerves, which has the same diameter as the first. An equal distance behind the second is situated the *third commissure,* which is the last and much the largest of the three, both in length and diameter. Behind this last commissure the oesophageal nerves diverge from each other, as described by Hubrecht and Bürger, each passing downwards and outwards to occupy a latero-ventral position in the inner longitudinal muscle layer of the body wall, close to the oesophagus; in their course they give off a number of smaller nerves. The oesophageal nerves, both in the fibrous core of the brain as well as posteriorly, are enveloped by a neurilemmatic sheath, beneath which a few inner neuroglia cells lie (Fig. 43, *Oes. N.*). Ganglion cells are present only around the commissures of the nerves, most abundantly (though only in a single layer) around the third; these all are modifications of cells III of the brain lobes and have been already described (Fig. 20). The neurilemmatic sheath is absent only around the commissures.

In both species of *Lineus* examined the oesophageal nerves have four commissures, of which the fourth (last) is the largest, and accordingly corresponds to the third of *Cerebratulus*. The commissures are more widely separated in *Lineus;* otherwise the oesophageal nerves have the same structure as in *Cerebratulus*.

VII. General Conclusions.

1. To the central nervous system of the nemerteans belong the dorsal and ventral brain lobes, the lateral chords, the oesophageal and proboscidean nerves, and the dorsal, unpaired, longitudinal nerves.

2. All ganglion cells are membraneless and unipolar, and may be naturally divided into the four categories adopted by Bürger ('90b).

3. In all the ganglion cells one or two (never more) spherical nucleoli are present, which are readily distinguishable from the chromatin masses of the nucleus ; in the latter an achromatic reticulation is also present. I found only one cell with two nuclei.

4. The cytoplasm of the ganglion cells consists of staining, more or less granular, but not fibrillar, spongioplasm, and unstaining, homogeneous hyaloplasm, the latter usually in excess, especially in the distal portion of the cell. The cell is bounded by a thin peripheral layer of fine-grained spongioplasm not in connection with the external neuroglia, and a similar layer envelops the nucleus ; these probably represent superficial alveolar layers, and the structure of the cytoplasm a honeycombed meshwork, in the sense of Bütschli ('94).

5. In the cytoplasm of cells III in *Lineus*, homogeneous, deeply staining, more or less spherical bodies occur, which are never numerous, and have no regular arrangement in the cell ; to these bodies, which cannot be classed with the chromophilic granules of other forms, the term *chromophilic corpuscles* has been applied here.

6. The axis-cylinder process is the same in all the ganglion cells ; it represents a nerve tubule and not a "nerve fibril," and is composed of : (1) the homogeneous, unstaining axis

cylinder, which is probably of a fluid (or at least a viscid) consistency in life, and of (2) a fine spongioplasmic sheath enveloping the latter. The hyaloplasm of the axis cylinder is in direct connection with that of the cell, and its spongioplasmic sheath with the peripheral spongioplasmic layer of the latter. "Primitive nerve fibrils" are absent in the homogeneous axis cylinder, and the nerve tube is not composed of a bundle of separate "primitive nerve tubes" (Nansen), though in a few cases irregular strands of spongioplasm may penetrate from the cell into the most proximal portion of the axis cylinder. The nerve tubule gives off fine, unbranching collaterals, and decreases in diameter distally; it does not terminate in a number of brush-like processes. There is no structural difference between the proximal and distal portions of the nerve tubule, *i.e.*, no structural distinction into a proximal "ganglion-cell process" and a distal "nerve fibril."

7. The colossal nerve tubules of *Cerebratulus*, the so-called neurochords, differ from the other tubules (1) in their superior size; (2) in that they do not give off collaterals but divide dichotomously, the two branches formed by such division being of equal size; (3) in their segmental constrictions.

8. I found no substance resembling myelin in the nervous system.

9. The colossal ganglion cells (IV) of *Cerebratulus* (absent in *Lineus*) are present in three pairs in the ventral brain lobes, and are distributed irregularly along the lateral chords, but are wholly absent in both ends of the latter (namely, in the oesophageal region and in the caudicle). In the lateral chord they increase in number posteriorly, and are dorsally more abundant than ventrally; those in the one chord do not regularly alternate with, nor are they paired with, those in the other. The only apparent regularity in the distribution is that areas where they are comparatively numerous alternate with zones where they are relatively scarce.

10. In each lateral chord of *Cerebratulus*, but apparently not of *Lineus*, both dorsally and ventrally the radial clusters of cells II show a bilateral arrangement.

11. Ganglion cell III has structural affinities on the one hand to II, on the other to IV ; these three are probably motor cells.

12. In the fibrous core representing the so-called dotted substance the following components may be distinguished : (1) staining, dense, branching fibres of the inner neuroglia cells, these being the only fibrillar constituents of the dotted substance ; (2) nerve tubules and their sheaths ; (3) irregular, branching spaces filled with body fluid. In the lateral chord of *Lineus* is situated a longitudinally directed *nerve tube* near the center of the fibrous core, composed of a large number of individual nerve tubules. This nerve tube gives off smaller nerve tubes which pass to the peripheral nervous system ; anteriorly it is not continued into the brain. By no means all, probably not half, of the nerve tubules in the fibrous core are enclosed in this nerve tube, since a large number are distributed pretty evenly throughout the fibrous core.

13. The fibres of the outer neuroglia cells produce not only loose sheaths around the ganglion cells III and IV, but are also continued distally to form a sheath of Schwann around the nerve tubule ; this sheath, which is never occupied by nuclei, is not a continuous membrane, but apparently consists of a single layer of parallel fibres, which are separated from one another. The neuroglia of the nemerteans is fully comparable to that of the annelids or vertebrates, and stands in no relationship to the elements of the neurilemmatic sheaths.

14. In *Cerebratulus* and *Lineus gesserensis* there is a second ventral commissure of the brain uniting the ventral lobes, situated behind the first massive commissure, and of much smaller diameter. In *L. sp.* there is, in addition to the second, also a third ventral commissure.

15. In *Cerebratulus* there are three commissures of the oesophageal nerves, the third (most posterior) being the largest. In *Lineus* there are four such commissures, the fourth of which is the largest, corresponding to the third of *Cerebratulus*.

At the close of this brief study of the elements of the central nervous system of *Lineus* and *Cerebratulus* it is not my intention to enter into a discussion of the many views in regard to

the structure of the nervous elements in general; any one wishing to consult the rather voluminous literature on this subject may find more or less complete bibliographies given by H. Schultze ('79), Eisig ('87), Nansen ('87), Rohde ('90), and Friedländer ('89). But one point may be mentioned, namely, that the newer researches are tending to prove that the nervous element of the so-called "dotted substance" is a *nerve tubule* and not a *nerve fibril;* and that the fibrillar elements of this substance are derivatives of the neuroglia, or of other connective tissues. Whether, however, as Leydig ('85) maintains, the hyaloplasm alone represents "die eigentliche Nervenmaterie," we are not as yet in position to decide; for the nerve tubule consists not only of hyaloplasm (though this substance may preponderate), but also of spongioplasm. And unless one would side with Rohde ('90a, 92) in assuming — what seems very improbable — that the spongioplasm of the ganglion cell is formed entirely by neuroglia fibres, we must consider the spongioplasm as much a vital substance of the cell as the hyaloplasm, and therefore as much a vital part of the nerve tubule. Further, the dotted substance would appear not to be a "spongy meshwork" with confluent meshes, but to consist of nerve tubules (which do not anastomose), between which a fibrous neuroglia network is situated.

The structure of the axis cylinder has also been a subject of much controversy, and has been described (1) as homogeneous, (2) as fibrillar, (3) as consisting of a number of parallel "primitive nerve tubes," (4) as having a honeycombed meshwork structure. This difference of opinion is due to two reasons: (1) the study of different objects, and (2) the use of different technical methods on the part of the observers; for seldom has an investigator studied a number of different organisms and at the same time employed various reagents. Now because I have found the axis cylinder of the nemerteans to be homogeneous, it would be false reasoning for me to conclude that this structure holds good also in other forms. On the contrary, the researches on the vertebrate axis cylinder would show it to be fibrillar (with "primitive fibrils" enclosed in hyaloplasm); while in some of the invertebrates it would seem to be fibrillar,

in others homogeneous. *A priori* we would expect the axis cylinder, as any other organ, to be more highly differentiated in the higher forms than in the lower; and the facts would seem to be, indeed, that in some of the invertebrates it is homogeneous, while in the (higher) vertebrates it may be fibrillar. And at any rate it is always unsafe to conclude that if the axis cylinder, or any other nerve element, has a particular structure in one organism, the same structure must exist also in but distantly related forms.

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EXPLANATION OF PLATES.

The outlines of all figures, with the exception of the diagram 26, have been drawn with the camera lucida of Zeiss; and unless otherwise stated, have been made with homogeneous immersion $\frac{1}{12}$ of Zeiss, with ocular 4. The following abbreviations have been employed in the figures:

Achr.	achromatic substance.
Alv.	alveolar layer.
Ax. Cl.	axis cylinder.
Ax. Cl. IV	neurochord.
C. II	ganglion cell II.
C. IV	ganglion cell IV.
Chr.	chromatin.
Cl. II	cluster of cells II.
c. Musc.	circular musculature.
Coll.	collateral of nerve tubule.
Comm. 2	second ventral commissure of brain.
D.	dorsal.
D. V.	dorsal blood vessel.
F.	spongioplasmic fibre.
F. C.	fibrous core.
G. C.	ganglion-cell layer.
Gr.	chromophilic corpuscle.
Hl. Pl.	hyaloplasm.
i. Neur.	inner neurilemma.
L.	lateral.
l. Musc.	longitudinal musculature.
L. V.	lateral blood vessel.
M.	medial.
Musc.	musculature.
N.	nucleus.
n.	nucleolus.
Ngl.	neuroglia.
Ngl. C.	neuroglia cell.
N. S.	nuclear sap.
N. T.	nerve tube.
Oes. N.	oesophageal nerve.
o. Neur.	outer neurilemma.
P.	pore (opening) of inner neurilemma.
Prob.	proboscis.
Sp.	space containing body fluid.
Sp. Pl.	spongioplasm.
S. Sh.	sheath of Schwann.
V.	ventral.

EXPLANATION OF PLATE XXIV.

FIG. 1. *Lineus gesserensis:* ganglion cell I. Sublimate, Del. haematoxylin + eosin.

FIG. 2. *Ibid.:* ganglion cells II. Sublimate, Del. haematoxylin + eosin.

FIGS. 3–10. *Ibid.:* ganglion cells III. Sublimate, Del. haematoxylin + eosin.

FIGS. 11, 12. *Ibid.:* ganglion cells III. Sublimate with acetic acid, Ehrl. haematoxylin + eosin.

FIG. 13. *Ibid.:* ganglion cell III. Flemming's fluid, Del. haematoxylin + eosin.

FIG. 14. *Ibid.:* ganglion cell III. Hermann's fluid, Ehrl. haematoxylin + eosin.

FIGS. 15, 16. *Lineus sp.:* portions of ganglion cells III. Sublimate, Del. haematoxylin + eosin.

FIG. 17. *Cerebratulus lacteus:* The two modifications of ganglion cells I. Sublimate, Del. haematoxylin + eosin.

FIG. 18. *Ibid.:* ganglion cells II, from the ventral side of the ventral brain lobe. Sublimate, Del. haematoxylin + eosin.

FIG. 19. *Ibid.:* an unusually large ganglion cell III from the dorso-ventral brain commissure. Sublimate, Del. haematoxylin + eosin.

FIG. 20. *Ibid.:* ganglion cell III from third commissure of the oesophageal nerves. Sublimate, Del. haematoxylin + eosin.

FIG. 21. *Ibid.:* ganglion cell III with two nuclei, from lateral side of ventral brain lobe. Sublimate, Del. haematoxylin + eosin.

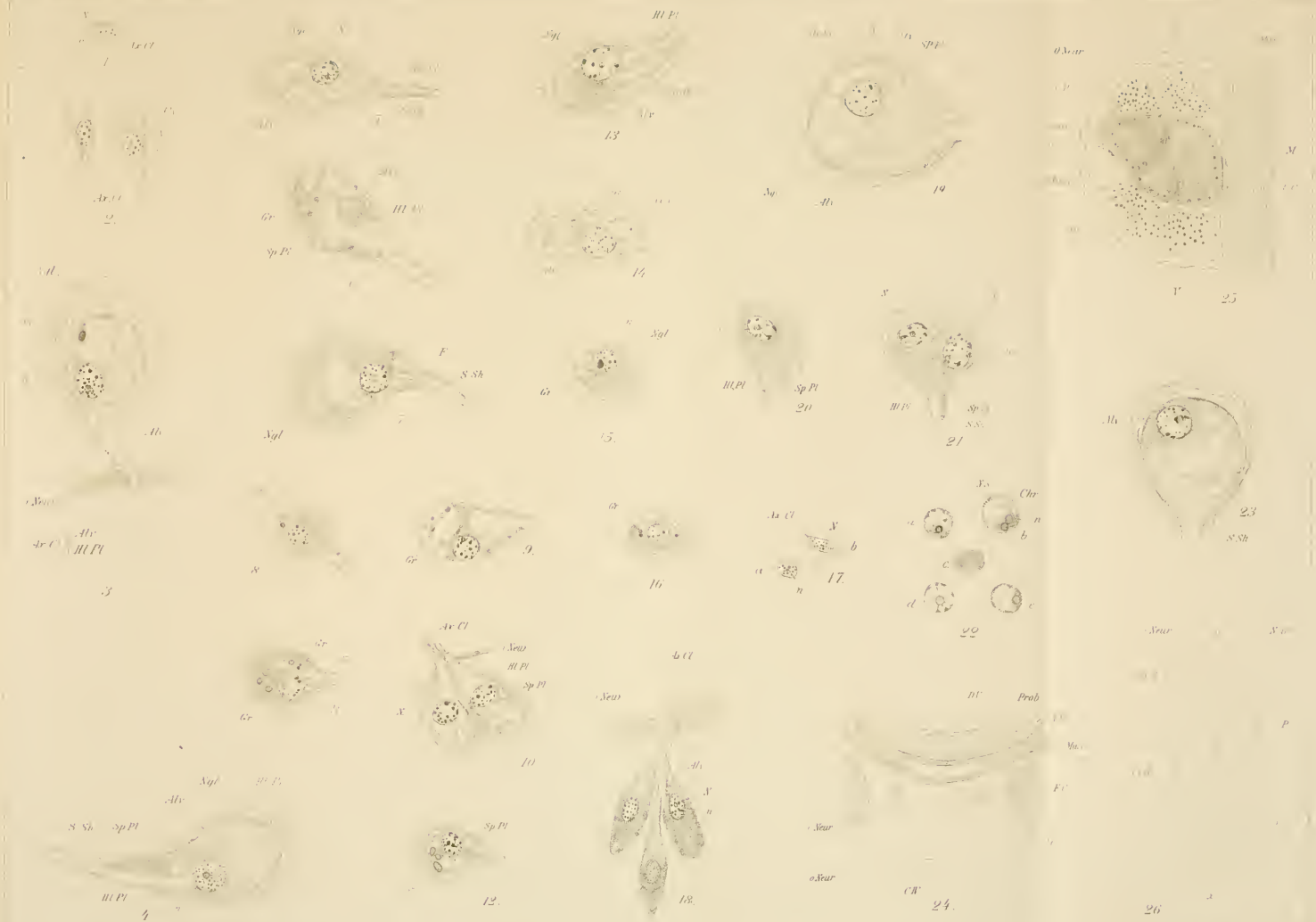
FIG. 22*a–e*. *Ibid.:* nuclei of ganglion cells III. Sublimate, Del. haematoxylin + eosin.

FIG. 23. *Ibid.:* ganglion cell intermediate between III and IV, from the ventral side of the lateral chord. Sublimate, Del. haematoxylin + eosin.

FIG. 24. *Ibid.:* part of a cross section of the ventral lobe of the brain, showing the position of the first pair of ganglion cells IV of the brain. Obj. A, oc. 4.

FIG. 25. *Ibid.:* cross section of a lateral nerve chord, close behind the mouth region, showing radial clusters of ganglion cells II. Obj. C, oc. 2. Sublimate, Del. haematoxylin + eosin.

FIG. 26. *Ibid.:* diagram to illustrate the regular arrangement of the cell clusters of cells II (*Cl. II*) and their respective openings (*P.*) in the inner neurilemma, on either the dorsal or the ventral side of the lateral nerve chord; *X–X*, the imaginary median plane of symmetry.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EXPLANATION OF PLATE XXV.

FIGS. 27, 28. *Cerebratulus lacteus:* ganglion cells IV (neurochord cells) from the lateral chord. Sublimate, Del. haematoxylin + eosin.

FIG. 29. *Ibid.:* ganglion cells III and IV, from the dorsal side of the lateral chord. Sublimate, Del. haematoxylin + eosin.

FIG. 30. *Ibid.:* portion of a horizontal, longitudinal section of the fibrous core of the lateral nerve chord, from the middle body region. Sublimate, Del. haematoxylin + eosin. Obj. C, oc. 4.

FIG. 31. *Ibid.:* Nuclei of ganglion cells IV. Sublimate.

a, *b*, from the lateral chord. Ehrl. haematoxylin + eosin.

c, *d*, of the right and left cells IV of the first brain pair. Del. haematoxylin + eosin.

e, from the lateral nerve chord. Del. haematoxylin + eosin.

FIG. 32. *Ibid.:* two contiguous ganglion cells IV, from the lateral chord; their respective nerve tubules not situated in the same plane. Sublimate, Del. haematoxylin + eosin.

FIG. 33 *a–c*. *Lineus gesserensis:* outlines of the nerve tube of the lateral nerve chord on four consecutive cross sections, to show its manner of branching. Hermann's fluid.

FIG. 34. *Ibid.:* outline of a cross section of the fibrous core of the lateral nerve chord, to show the position of the nerve tubes (*N. T.*). Hermann's fluid. Obj. C, oc. 4.

FIG. 35 *a–c*. *Cerebratulus lacteus:* transverse sections of the right lateral nerve chord. Obj. C, oc. 4. Sublimate, Del. haematoxylin + eosin.

a, from the oesophageal region.

b, behind the middle body region.

c, from the anterior portion of the caudicle.

FIG. 36. *Lineus gesserensis:* cross section of the fibrous core of the lateral chord, in the anterior oesophageal region. Hom. immers. $\frac{1}{12}$, oc. 2. Hermann's fluid, Ehrl. haematoxylin + eosin. In this, and in Figs. 37, 38, the appearance of the preparation has been reproduced, the white lines representing the unstained axis cylinders, and the dark ground (which should be of a bronze color) the deeply staining, non-nervous substance of the fibrous core; seen with this magnification, this staining matrix appears nearly homogeneous.

FIG. 37. *Ibid.:* cross section of the fibrous core of the dorsal brain lobe. Hom. immers. $\frac{1}{12}$, oc. 2. Hermann's fluid. (Cf. explanation of Fig. 36.)

FIG. 38. *Ibid.:* frontal section of the fibrous core of the first ventral commissure of the brain. *a*, the branch towards the dorsal lobe; *b*, that to the ventral lobe. Hom. immers. $\frac{1}{12}$, oc. 2. Hermann's fluid. (Cf. explanation of Fig. 36.)

FIG. 39. *Ibid.:* portion of a thin cross section of the fibrous core of the dorsal brain lobe. Hom. immers. $\frac{1}{12}$, oc. 4, tube extended. Hermann's fluid $\frac{1}{2}$ hour, Ehrl. haematoxylin + eosin.

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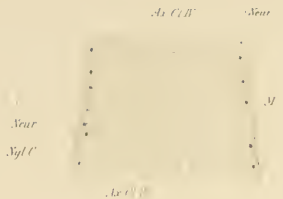

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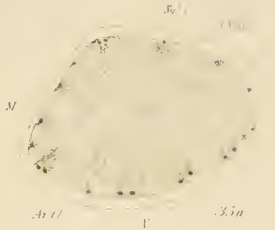

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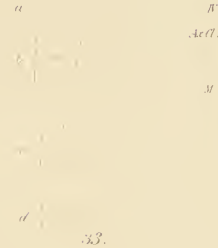

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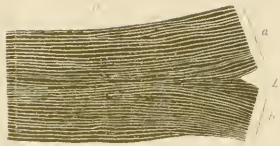

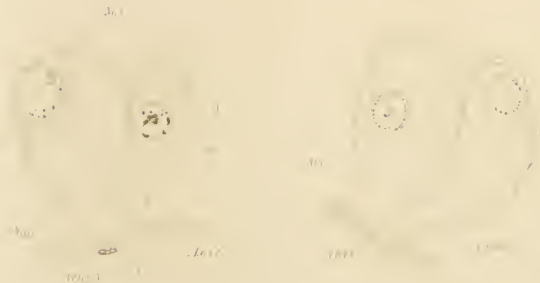

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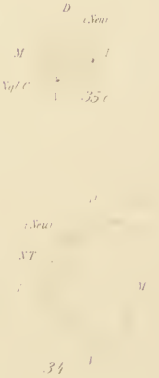

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EXPLANATION OF PLATE XXVI.

FIG. 40. *Cerebratulus lacteus:* segment of a cross section through the lateral side of the fibrous core of the lateral nerve chord, from about the middle body region. Sublimate, Ehrl. haematoxylin + eosin.

FIG. 41. *Tetrastemma vermiculum:* portion of a horizontal, longitudinal section of a lateral nerve chord (only the outlines drawn), showing a supposed nerve tube (*N. T. ?*) stained with methylene blue. Sublimate, aq. sol. of methylene blue 10 minutes, aq. sol. of brasilin 40 minutes.

FIG. 42 *a–e. Cerebratulus lacteus:* portions of longitudinal sections of neurochord tubules, from the fibrous core of the lateral nerve chord, behind the middle body region. Sublimate, Del. haematoxylin + eosin.

FIG. 43. *Lineus gesserensis:* portion of a cross section of the posterior ventral portion of the brain, showing the second ventral commissure. Obj. C, oc. 2. Hermann's fluid.

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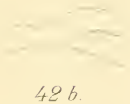

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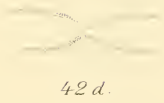

42 d.

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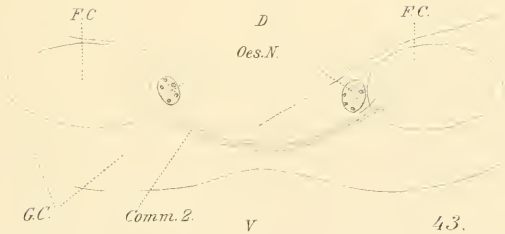

43.

A COMPARATIVE STUDY OF THE AREA OF ACUTE VISION IN VERTEBRATES.

JAMES ROLLIN SLONAKER,

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Introduction.

This investigation has been pursued during the past three years in the Neurological Laboratory of Clark University under the direction of Dr. C. F. Hodge, to whom I am under great obligations for his assistance and encouragement. I am also greatly indebted to Clark University for the apparatus and material which has made this work possible.

Up to the present I have been engaged chiefly in a gross comparison of the retina rather than in its minute histology, therefore my aim will be, first, to sum up the results of others and also to add my own; second, to correlate as far as possible the habits of the animal with its visual apparatus.

Since there are so many investigators who have written on various phases of the eye, it will be impossible to mention all. Reference, therefore, will be made to only a few of the most important in the historical résumé and literature on the subject.

I have adopted the nomenclature of the German investigators and called the structure corresponding to the macula lutea of man the *area*. According to the position of the area or fovea on the nasal or temporal side of the optic nerve entrance, it is called *area* or *fovea nasalis* or *temporalis*.

Historical.[1]

On the basis of the methods of investigation employed, the literature may be divided into three periods: (1) from the ear-

[1] The literature on this subject has been fully presented by J. H. Chievitz (Ueber das Vorkommen der Area centralis retinae in den vier höheren Wirbelthierklassen, Arch. f. Anat. u. Entwickelungsgeschichte, 1891, Heft 4, 5, u. 6, pp. 311–321), but as I have not found it anywhere in English, I will devote some space to it.

liest investigations till about 1830, or previous to the common use of the microscope; (2) from the use of the microscope till 1887, or a period when the old methods of hardening and staining were employed, which made only the nuclei and larger processes visible; and (3) from 1887 to the present time, or since the use of the silver chromate and the methyl-blue methods of staining, which make clear not only the cells, but the finest processes of both neurites and dendrites.

Although Francesco Buzzi (3) is given the credit of having discovered the yellow spot in the human eye in 1782, it was not until 1791 that the fovea centralis was noticed. This discovery was made by the celebrated German anatomist, Sm. Th. v. Soemmerring (2), and was called for a number of years the "Foramen of Soemmerring," he having considered it a perforation. Buzzi, on the contrary, thought it merely a thin and transparent part of the retina. Michaelis (4) favored Buzzi's theory, while Reil (5), Meckel (6), and Home (7) considered it a foramen.

The discovery of the foramen of Soemmerring in man naturally led to many investigations in other classes of vertebrates. Michaelis examined the eyes of the dog, swine, and calf, but found no trace of a fovea. Home (7), however, was more fortunate. Knowing the great similarity which existed in the anatomy of man and the monkey family, he wisely chose one of the latter, and consequently was the first to discover the fovea in the ape in 1798. He considered it a real foramen for the passage of a lymphatic vessel, and tried to correlate it with such a vessel in the optic nerve of the sheep and calf. Cuvier (8) confirmed the presence of the fovea in the ape family, but he considered it a thinning of the retina. This view gained ground, but it was not firmly established till 1830, when v. Ammon (9) demonstrated by the aid of the microscope that the retina was continuous through the fovea.

Albers (10) found in 1808 "a central hole surrounded by a yellow border" in the giant tortoise (Testudo mydas), but was not able to confirm such an appearance in the second eye.

Knox (11) in 1823 was the first to demonstrate the presence of a fovea in animals other than the primates. He examined

the eyes of some reptiles and demonstrated the presence of a well-defined fovea in the lizard (Lacerta scutata) and the chameleon. Joh. Müller (12) says: "A foramen centrale is present in the middle point of the retina of other reptiles, which is not visible as in man, where the limiting membrane is unharmed, but where the choroid shows through." H. Müller (13), who so admirably describes the eye of the chameleon and the retina of the different classes of vertebrates, describes a well-defined fovea found in many birds, while in some birds two foveæ are present. He also states that in mammals an area centralis is present, which approaches in structure the yellow spot of the human retina.

Three things were always sought for by the early investigators: the yellow spot, the foramen, and the folds of the retina, which more or less concealed the foramen. Though Home had described these folds as due to *post mortem* changes and not present in the fresh eye, they were considered as normal by many writers even as late as the middle of the present century (1).

The old theory that the fovea was a foramen which enlarged and contracted with the intensity of the light, thus protecting the retina from injury, rapidly gave place with the use of the microscope to the opposite view, that it was the place of acute vision. The microscope further brought out the fact that the cells of the yellow spot had a definite arrangement, and that this arrangement might be present without a fovea. With this thought in view investigations were made in all classes of vertebrates with the result that a fovea has been found in each class, and that an area centralis is quite common.

Hulke (14) has described the presence of a point in the retina of several amphibians and reptiles which, owing to a similar arrangement of cells, he thinks corresponds to the human fovea. Gulliver (15) has described the presence of a fovea in the fish, and Carrière (16) in Hippocampus. Hoffmann (17) describes an arrangement in the crocodile which corresponds to that in the fovea. Krause (18) treats of the eyes of different vertebrates, and states that the dove and cat possess a fovea, while the chicken and dog do not. He seems to be the only person

who has found a fovea in the cat. Ganser (19) and Chievitz (31) have found only an area.

Chievitz has described and pictured the area and fovea centralis in many animals, and put his results in a concise tabulated form. Other investigators of this period will be mentioned later in a similar tabulation.

Many obscure points were made clear by these numerous investigators. However, two points still remained unsolved: the structure of the molecular layers and the endings of the fine branches of the retinal cells. The solution of these points depended on a new method of research. This new method was inaugurated by Tartuferi (21) in 1887, who used the quick method of Golgi and succeeded in showing that the cell processes end in more or less fine tufts which did not anastomose with other bunches. Later on he discovered and described the structure of the molecular layers.

Dogiel (22) in 1888 so modified the Ehrlich method that it would stain the fresh retina. He was thus able to confirm almost all the results of Tartuferi. He found that the branches from different cells anastomose, but other investigators have not confirmed this. The works of Baquis (23) and Ramon y Cajal (24), who used the Golgi method, in general confirm the results previously obtained. Ramon y Cajal has made clear the endings of the rod and cone fibres in the outer molecular layer. He finds that there are certain cells of the inner nuclear layer which are related to the cones. That is, their terminal branches come in contact only with the processes from the cones, while other cells of this layer send their dendrites to the rods. In general, each cell communicates with many rods or cones, excepting in the fovea, where the process from each cell branches very little and comes in contact with but one cone.

Methods.

As I have only attempted a gross comparison of the areas of acute vision in this study, I have used only those hardening fluids and methods of research which will preserve the eye with the least possible distortion. For fine histological study of the

retinal cells, other methods such as that of Golgi or Ehrlich are preferable.

For my purposes it is necessary to obtain the eye fresh, at least not later than an hour after death, and subject it to the action of certain hardening fluids which will permeate and preserve without distorting the eye. *Post mortem* changes occur in the retina very soon, such as wrinkling in the neighborhood of the fovea, which obscure its shape and size and make sections through it of little value. The eye is carefully oriented in every case before it is removed from the head by sewing a small tag to the outer layers of the sclerotic (Fig. 1). In no

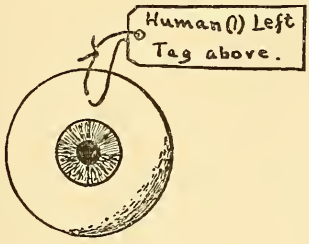

FIG. 1.

case should the eye be punctured in removal, for this invariably causes wrinkling of the retina and distortion of the ball.

I have tried many hardening fluids, but find that Perenyi's fluid works the best. It not only preserves the eye with little distortion, but also decalcifies all bone, thus making sections even through the whole head with eyes *in situ* possible. The different per cents of formaline which I have used have not proved satisfactory, as they caused wrinkling of the retina.

The former injection method[1] is now wholly replaced by that of simple immersion, which is as follows: after the eye is properly tagged, it is carefully removed and immersed for from 24 to 36 hours in Perenyi's fluid. The time depends upon the size of the eye and the amount of bone to be decalcified. It is then changed to 70% alcohol, and allowed to remain 24 hours. Quite frequently when this change is made the ball caves in and becomes somewhat distorted. This may be prevented or

[1] American Naturalist, January, 1896, p. 24.

the eye again made perfect by injecting into the vitreous chamber with a hypodermic syringe enough 70% alcohol to fill out the eye. It is kept 24 hours in each of the following liquids: 80, 90, 95, 100% alcohol, and a mixture of absolute alcohol and ether (one part each).

The eye is now well hardened and the front half may be cut off, leaving the posterior half uninjured. After the hardened vitreous humor is removed the retina is exposed to view. The entrance of the optic nerve, area and fovea centralis, if present, and the larger blood-vessels will be easily seen. In many cases the area is very indistinct and the blood-vessels wanting or so meagre as to be invisible to the naked eye.

When one wishes to section the eye, a window is cut in the same plane of the desired sections and the hardened vitreous humor carefully removed without injury to the retina or other structures. It is then changed to celloidin. Best results are obtained when three grades of celloidin are used: (1) very dilute; (2) less dilute; (3) as thick as will run. It is allowed to remain from four to six days in the first, six to eight days in the second, and ten to fifteen days or longer in the third. It is then mounted on a block and cut in 80% alcohol. In every case when sufficient material was at hand sections were made in vertical and horizontal planes. Serial sections were always saved through the fovea, so that the central section could be readily distinguished. Sections were stained in hæmatoxylin and eosin and mounted in balsam.

In order to demonstrate more quickly the presence or absence of an area or fovea centralis, the whole head of small

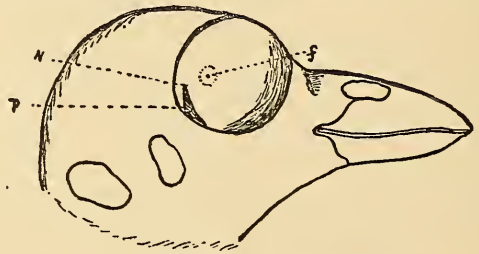

FIG. 2. — Snow-bird (Junco hyemalis) 3/1.

f. Fovea centralis. *N.* Nerve entrance. *P.* Pecten.

animals was immersed in the Perenyi for from three to six hours, when the anterior part of the eye was hardened so that the cornea, lens, and vitreous humor were easily removed, leaving the posterior half *in situ*. Better results are obtained when the skin is removed from the head before immersion.

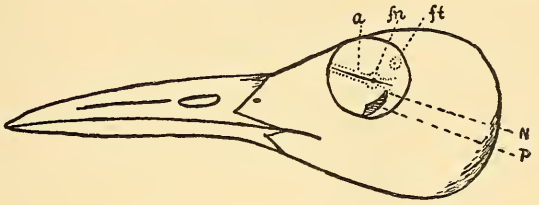

FIG. 3. — Tern (Sterna hirundo) $^1/_1$.

N. Nerve entrance. *a*. Band-like area. *ft*. Area and fovea temporalis.
P. Pecten. *fn*. Area and fovea nasalis.

With birds I have had good results, the retina lying back smoothly so that the fovea and entrance of the optic nerve, marked by the pecten, may be easily seen. Figs. 2 and 3 represent the appearance of the retina after the front of the eye has been removed. With other animals, especially mammals, there is a greater tendency for the retina to wrinkle.

Permanent demonstration material may be prepared by subjecting the whole head to the different fluids as described for the hardening of the eye. It is not necessary, however, to carry it farther than 80% alcohol when the front half of the eye and vitreous humor may be removed. Such material is preserved in 80% alcohol.

Sections were made through the whole head of several animals (fishes, amphibians, reptiles, birds, and some small mammals) in order to determine approximately the angles which the lines of vision make with the median plane. The plane of section passed through each fovea or center of area centralis and the center of the pupil. Fig. 4 represents such a section through the foveæ, *f*, *f*, of a chickadee's head (Parus atricapillus), while the lines *GH* and *GI* show the axes of vision. The axes of vision, owing to the mobility of the eye,

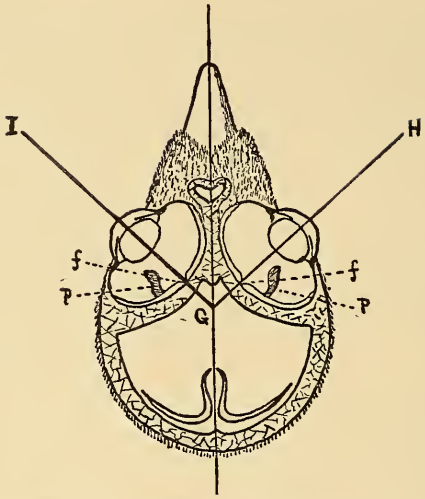

FIG. 4. — Chickadee (Parus atricapillus) 3/1.

f Fovea. *p* Pecten. *GH* and *GI* Axes of vision.

may be greater or less during life. The pecten, *p*, marks the entrance of the optic nerve. When a second fovea is present it is situated on the temporal side of the nerve entrance, as shown in Fig. 5.

In order to show the relation of the retinal arteries to the area and fovea centralis, they were injected with the gelatine-carmine mass of Ranvier. In small animals this injection was made in the carotid arteries, while with large animals the eyes were removed and the injection made into that branch of the ophthalmic artery which supplies the retina. After injection the eye was at once cooled and hardened in alcohol. When hardened, the front half of the globe and the vitreous humor were carefully removed, exposing to view the retina, arteries, entrance of nerve, and area and fovea centralis, when present. Usually the fovea is readily seen if it is present, but the area is sometimes very difficult to discern, and were it not for the blood-vessels acting as landmarks, it might be overlooked altogether. Drawings were made of the posterior half, great care being taken to orient it so that one would look into it

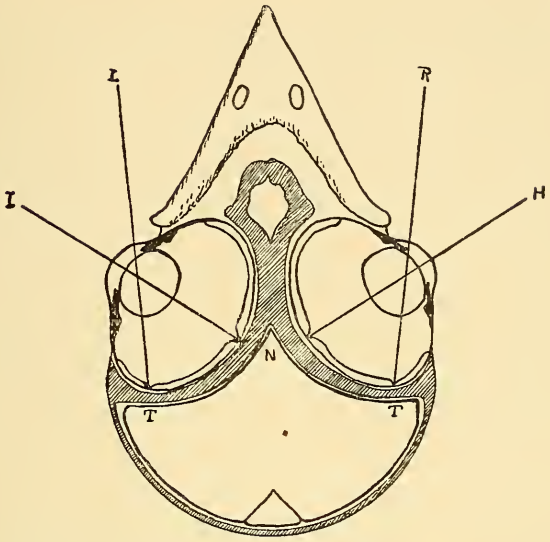

FIG. 5. — White-bellied Swallow (Tachycineta bicolor) 3/1.

NH and *NI*. Axes of vision of fovea nasalis.
TR and *TL*. " " " temporalis.

along the axis of vision. I have not attempted an exact representation of the area, but have only indicated by dotted lines its position and extent as I have found it (Pl. XXVII, Figs. 3, 4, 8, 9).

The results of these injections only serve to substantiate Müller's observations (25). He states that mammals are the only class of vertebrates which possess, in the true sense, a retinal circulation, while in many mammals only a meagre circulation is present (horse and rabbit). Denissenko (32) has found an exception to this statement. He describes and pictures retinal blood-vessels in the eel, which penetrate to the outer nuclear layer. This is the only exception which I have so far been able to find. In the sections which I have of the eel's eye, owing to their thickness I have not been able to demonstrate the presence of capillaries beyond the inner boundary of the outer nuclear layer. Fishes and amphibians possess a good circulation in the hyaloid membrane, while birds

and many reptiles have the circulation of the pecten. Huschke (26) states that these vessels of the hyaloid membrane and the pecten correspond to the retinal vessels in mammals. They do not, however, penetrate the retina.

The photographic representations of the fovea and area of the different animals were all taken with the same magnification so that they are directly comparable. In every case the section through the center of the fovea was selected. In some cases the section includes not only the bottom of the fovea, but also some cells of the inner edge of the area beyond. In this case the bottom of the fovea is more or less obscured by these cells. In case the retina does not lie smoothly on the choroid, its position is to be considered abnormal and due either to *post mortem* changes or to the reaction of the reagents.

DESCRIPTION.

Before giving a description of the areas of acute vision in the animals examined, a few words may be necessary regarding the development, form, position, and prevalence of the area and fovea centralis in different vertebrates.

In the development of a fovea an area centralis is first differentiated (27). This stage, according to Chievitz, is present in the human fœtus about the sixth month, after which time the fovea begins to appear (29). This takes place by a pitting in of the vitreal surface, or a crowding to the sides, as it were, of the cells in the center of the area. The area differs from the rest of the retina either in thickness or in compactness of cells. In some cases the difference in thickness is easily seen and sharply marked off (Pl. XXVII, Figs. 1, 3, 6, 7, 8), while in others the increase is very gradual and slight (Pl. XXVII, Fig. 5). The increase in thickness is due usually to a greater number of nerve cells, cells of the inner and outer nuclear layers, and greater length of the rods and cones. But an area is not necessarily designated by an increase in thickness of any of these layers because the cells may be more numerous and packed more closely together. This is well illustrated in the nerve-cell layer of the frog's area, which is but a single cell

deep over the entire retina. In the center of the area, however, the cells lie closely together, while in other parts of the retina they are some distance apart.

The rods and cones in the area have a less diameter and are more numerous per given area than elsewhere. Hence the cells which form their connection with the nerve fibres (nerve cells and cells of the inner nuclear layer, or bipolar cells) must also be more numerous. But this is not the only reason for an increase in the number of cells in the area. Ramon y Cajal (28) has described and pictured the manner in which these cells form the connection between the nerve fibres and the rods and cones. Numerous processes (dendrites) from the nerve cells pass outward and branch profusely in the inner molecular layer among the ingoing branches (neurites) of the cells of the inner nuclear layer. A similar relation exists in the outer molecular layer between the outgoing branches (dendrites) of these cells, which are bipolar, and the ingoing branches (neurites) of the cells of the outer nuclear layer. These fine branches from the cells of different layers only come in close relation, and in no case were they found to anastomose with each other. He divides these bipolar cells into two classes: (1) those whose dendrites come in contact with the neurites from the cone cells; and (2) those whose dendrites come in contact with neurites from the rod cells. In the periphery of the retina each bipolar rod cell may come in contact with from 10 to 30 rods. Likewise each bipolar cone cell is related to several cones. But toward the center of vision the number of rods and cones which connect with a single bipolar cell becomes rapidly less, and in the center of the fovea each bipolar cell comes in contact with but a single cone. Ramon y Cajal also finds a similar relation existing between the cells of the nerve-cell layer and the bipolar cells of the inner nuclear layer.

The number of cells in the outer nuclear layer is directly dependent on the number of rods and cones, each rod and cone having but a single nucleus. In fact a rod, or cone, with its nucleus, has long been considered as a much drawn-out cell whose dendritic end (the rod or cone) is more or less distant from the nucleus, and is in some cases connected only by a

fibre. In the periphery of the retina each rod and cone nucleus lies vertically over the rod or cone to which it belongs, and the cone nucleus is in the base of the cone. But in the region of the fovea these nuclei are crowded toward the periphery and lie some distance from the rods and cones to which they belong. This causes a diagonal appearance of these fibres in a cross section of the retina. The cone nuclei here lie three or four deep. The rods gradually decrease in relative number toward the area or fovea, and in the center of the fovea they are wanting, cones only being present. Borysiekiewicz (30) states, however: "Within the fovea centralis all distinguishing characteristics between the rods and cones are lacking; the so-called cones of the fovea entirely resemble the rods in the periphery of the retina, it is therefore correct to speak, not of the cones, but of the rods of the fovea." Most investigators do not uphold his view.

The length of the rods and cones in the fovea and area varies considerably in different animals. They may be longer than in other parts of the retina, as in the crow (Pl. XXIX, Fig. 52), or shorter, as in the ring-neck plover (Pl. XXIX, Fig. 47). This difference in length of rods and cones is shown by a greater or less thickness of the pigment layer. Dimmer (29) thinks in the human macula the rod and cone layer is of about the same thickness as elsewhere.

If the front half and vitreous humor is removed from the hardened eye, in many animals the area is readily seen as a whiter region which has various shapes and positions. It is never sharply marked off from the surrounding retina, but gradually blends with it. Its form may be circular, oval, or band-like. In the latter case it may also contain a circular area, as in the ring-neck plover, goose, and tern (Pl. XXVII, Figs. 8, 12). The round area may be situated on the nasal side of the nerve entrance, in which case it is designated *area nasalis;* or it may be on the temporal side, and designated *area temporalis*. Usually at least one of these different kinds of areas is present, and all three may be present as in the tern. In many eyes, however, when examined in this macroscopical way, no area is visible. It is not until sections are made and subjected to careful microscopical examination and measure-

ment that an area is discerned. Even with the aid of the microscope the presence of an area is often very doubtful.

The fovea is also found to have a variety of forms and positions. In depth it may vary from the very questionable depression found in the guinea hen (Pl. XXVIII, Fig. 36) to the very deep pit of the crow or hawk (Pl. XXIX, Figs. 48, 50, 52). It has also been found to vary in the same species, but this may be due to slight swelling produced in hardening (pigeon, Pl. XXVIII, Figs. 37, 38). In size it varies from the very broad fovea in the human (Pl. XXVIII, Figs. 24, 25) to the exceedingly sharp depression in the sparrow hawk (fovea nasalis) and the horned toad (Pl. XXX, Fig. 56). Chievitz (31, *a*) claims that certain animals have also a trough-like fovea, such as the tern, etc. I have not been able to procure any of the species which he describes as having a trough-like fovea, but have succeeded in obtaining a species from the same genus, Sterna. In a macroscopical examination one would at once conclude that there was a trough-like fovea present, but when sections were made across this trough-like appearance, no depression was found (Pl. XXIX, Figs. 40, 42). In my researches I have seen nothing excepting the macroscopical appearance which might be taken to indicate the presence of a trough-like fovea in any of the animals examined. Chievitz has pictured only the macroscopical view of his trough-like fovea, and in no place have I found that he has made cross sections and microscopical examination. In the tabulation which follows I have used his descriptions, as I have not been able to examine the species which he has described.

The relations of the fovea correspond to the positions above described for the area. That is, we find a *fovea nasalis*, as in the crow, blue jay, robin, snow-bird, etc., and a *fovea temporalis*, as in man, gorilla, owl, tern, hawks, etc. One or two foveæ may be present, but in each case where two are present the nasal fovea is always deeper than the temporal.

A very noticeable and important difference in the position of the fovea in various birds has been observed. Very little variation is found in the position of the fovea nasalis, but the location of the fovea temporalis depends wholly upon the position

of the eye in the head. As the eyes are turned more and more forward, the fovea temporalis approaches the fovea nasalis, and as binocular vision becomes more frequent, the nasal fovea becomes less distinct or merges into the temporal. This change is shown in Plate XXXI. The change to an asymmetrical form, and the position of the lens in the eyes of the birds which possess the power of binocular vision, is also quite marked. In many of these birds, as the tern and white-bellied swallow (Pl. XXIX, Figs. 40, 41, 46, and Pl. XXXI, Figs. 64, 65), where the fovea nasalis is sharp and deep and the fovea temporalis quite shallow, the eyes are almost symmetrical. But in those birds which use binocular vision more as shown by a greater depth of the temporal fovea, as in the hawks (Pl. XXIX, Figs. 48–51, and Pl. XXXI, Figs. 66, 67), the eye becomes more asymmetrical, and finally reaches the most irregular form in the owl (Pl. XXXI, Fig. 68), where binocular vision only occurs.

Various combinations of area and fovea are found in different animals. The most simple is a single fovea surrounded by a circular area, as in the primates and most birds. Again we find a simple fovea surrounded by a round area which is continuous with a band-like area extending horizontally across the retina, as in the goose and ring-neck plover. One or two foveæ may be present, each surrounded by a round area and connected by a short, slight, band-like area, as in the sparrow hawk, red-tailed buzzard, and kingfisher. The most complex combination which I have found is in the tern. Here the fovea temporalis surrounded by a small round area is not connected with the band-like area which extends horizontally across the retina, and near its middle widens out into a round area surrounding the fovea nasalis (Pl. XXVII, Fig. 12).

In my researches I have been able to examine 93 different species, of which 18 were mammals, 41 birds, 6 reptiles, 3 amphibians, and 25 fishes. In some cases the results were doubtful, and sufficient material was not available to ascertain all points with certainty. Such cases I have indicated. Many of the species examined have been observed by others, in which case I have always aimed to give credit to the first observer.

In the animals which I have examined the space for the observer is left blank, excepting when the species has been previously examined, in which case I have inserted (S) after the name of the first investigator.

In the following tabulation I have adopted the form of Chievitz, and have endeavored to present the results of all investigators up to the present time. Some results have necessarily been omitted, as the investigators in their descriptions have given only the common name and not the species of the animal.

	No Area Found	Simple		More Complex		First Observer
		Area	Fovea	Area	Fovea	
MAMMALIA						
Primates						
Homo		round	deep			Buzzi (3) [1782]; Soemmerring (2) [1791](S)
Chrysothrix sciurea		round	medium			Soemmerring (34) [1818]
Cebus capucinus		round	medium			Albers (10) [1808]
Cebus apella		round	medium			Soemmerring (34) [1818]
Cynocephalus sphinx		round	medium			Soemmerring (34) [1818]
Cynocephalus niger (?)		round	medium			Cuvier (8) [1800]
Macacus cynomolgus		round	medium			Albers (10) [1808]
Inuus sylvanus		round	medium			Blumenbach (35) [1815]
Inuus (?)		round	medium			Soemmerring (34) [1818]
Cercopithecus sabæus		round	medium			Albers (10) [1808]
Satyrus orang		round	medium			Cuvier (8) [1800]
Simia petarvista		round	medium			Cuvier (8)
Gorilla engena		round	medium			
"Aygula"		round	medium			Soemmerring (34) [1818]
Simia Talapoin		round	medium			Albers (10) [1808]
Simia Capucina		round	medium			Albers (10)
Simia Cynomolgus		round	medium			Blumenbach (35)
Rodentia						
Lepus sylvaticus		band-like				
Lepus Europæus		band-like				Chievitz (31) [1891]
Lepus cuniculus		band-like				Chievitz

Cavia cobaya	o					Chievitz
Arvicola agrestis	o					Chievitz
Mus musculus (Albin)	o					Chievitz
Mus decumanus (Albin)	o					Chievitz
Mus rattus	o					
Arctomys monax		oblong				
Sciurus hudsonicus		oblong				
Sciurus niger		oblong				
Sciurus vulgaris						
Tamias striatus		oblong				Chievitz
Chiroptera						
Vespertilo subulatus	o					
Ungulata						
Ovis avies		round (?)				Schwalbe (36) [1887] (S)
Bos taurus domesticus		band-like				Chievitz (S)
Cervus capreolus		round				Chievitz
Camelus Bactriuaus		band-like				Chievitz
Sus domesticus		band-like				Chievitz (S)
Equus caballus		band-like				Chievitz (S)
Carnivora						
Phoca vitulina		round				Chievitz
Felis catus domesticus		round	{ round (Krause 18)			Ganser (19) [1882] (S)
Tigris regalis		round (?)				Borysiekiewicz (33) [1887]
Felis leopardus		round (?)				Borysiekiewicz (33) [1887]
Meles taxus	o					Chievitz
Mustela erminea		round				Chievitz

	No Area Found	Simple		More Complex		First Observer
		Area	Fovea	Area	Fovea	
Mephitis mephitica		oblong				
Putorius vison		oval (?)				
Vulpes vulpes		band-like				
Canis familiaris		round				Chievitz (S)
Canis vulpes		round				Chievitz
Insectivora						
Sorex vulgaris	o					Chievitz
Talpa Europæus	o					Chievitz
Erinaceus Europæus	o					Chievitz
AVES						
Pygopodes						
Fratercula Mormon				nasal band-like	deep shallow	Chievitz
Alca torda				nasal band-like	shallow o	Chievitz
Uria troile				nasal band-like	shallow shallow	Chievitz
Longipennes						
Larus canus				nasal band-like	deep shallow	Chievitz
Larus ridibundus				nasal band-like	deep shallow	Chievitz

Sterna hirundo				nasal temporal band-like	deep shallow o	
Sterna macrura				nasal temporal band-like	deep shallow shallow	Chievitz
Sterna minuta				nasal temporal band-like	medium shallow shallow	Chievitz
Sterna Cantiaca				nasal temporal band-like	deep medium shallow	Chievitz
Anseres						
Anas boschas domesticus				nasal band-like	shallow o	Chievitz (S)
Oidemia deglandi				nasal band-like	shallow o	
Anser cinerus domesticus				nasal band-like	shallow o	Chievitz (S)
Fuligula glacialis				nasal band-like	medium o	Chievitz
Herodiones						
Ardea cinerea		nasal	deep			Chievitz
Nycticorax nycticorax		r. nasal	deep			
Limicolæ						
Recurvirostra avocetta				nasal band-like	deep o	Chievitz

	No Area Found	Simple		More Complex		First Observer
		Area	Fovea	Area	Fovea	
Gallinago media		nasal	deep			Chievitz
Tringa Islandica				nasal band-like	deep shallow	Chievitz
Tringa alpina				nasal band-like	deep shallow	Chievitz
Ereunetes pusillus				nasal band-like	deep o	
Limosa Lapponica				nasal band-like	deep o	Chievitz
Totanus glareola				nasal band-like	deep o	Chievitz
Totanus hypoleucus				nasal band-like	deep shallow	Chievitz
Numenius arquata				nasal band-like	deep medium	Chievitz
Charadrius hiaticula				nasal band-like	(?) shallow	Chievitz
Charadrius pluvialis				nasal band-like	deep shallow	Chievitz
Squatarola Helvetica				nasal band-like	(?) shallow	Chievitz
Ægialitis semipalmata				nasal band-like	deep o	

Vanellus cristatus				nasal	medium	Chievitz
				band-like	shallow	
Strepsilas interpres				nasal	deep	Chievitz
				band-like	shallow	
Hæmatopus ostralegus				nasal	medium	Chievitz
				band-like	o	
Gallinæ						
Colinus virginianus		r. nasal	medium			
Bonasa umbellus		r. nasal	medium			
Perdix cinerea		nasal	medium			Chievitz
Phasianus Colchicus		nasal	medium			Chievitz
Meleagris gallopavo		nasal	shallow			Chievitz (S)
Gallus domesticus		nasal	o (?)			Chievitz (S)
Numida pucherani		r. nasal	shallow (?)			
Columbæ						
Columba livia domesticus		r. nasal	shallow			Englemann [1884] (S)
Raptores						
Buteo borealis				nasal	very deep	
				temporal	deep	
Buteo vulgaris				nasal	deep	Chievitz
				temporal	medium	
Falco sparverius				nasal	very deep	
				temporal	deep	
Strix noctua		temporal	deep			H. Müller (1861)
Strix otus		temporal	deep			Chievitz
Syrnium nebulosum		r. temporal	deep			
Megascops asio		r. temporal	deep			

	No Area Found	Simple		More Complex		First Observer
		Area	Fovea	Area	Fovea	
Coccyges						
Ceryle alcyon				r. nasal r. temporal	deep medium	
Pici						
Picus major		r. nasal	deep			Chievitz
Melanerpes erythrocephalus						
Colaptes auratus		r. nasal	deep			
Macrochires						
Cypselus apus				r. temporal band-like (?)	deep	Chievitz
Passeres						
Tyrannus tyrannus		r. nasal	deep			
Pici candata		nasal	deep			Chievitz
Garrulus glandarius		r. nasal	deep			Chievitz
Cyanocitta cristata		r. nasal	deep			
Sturnus vulgaris		nasal	deep			Chievitz
Corvus americanus		r. nasal	deep			
Corvus cornix		nasal	deep			Chievitz
Corvus frugilegus		nasal	deep			Chievitz
Agelaius phœniceus		r. nasal	deep			
Emberiza miliaria				nasal band-like	deep shallow	Chievitz
Emberiza citrinella		nasal	deep			Chievitz
Fringilla cœlebs		nasal	deep			Chievitz

Fringilla cannabina				nasal band-like	deep shallow	Chievitz
Fringilla Canaria		nasal	deep			Chievitz
Fringilla domestica		nasal	deep			Chievitz
Fringilla montanan		nasal	deep			Chievitz
Spinus tristis		r. nasal	deep			
Poocætes gramineus		r. nasal	deep			
Spizella pusilla		r. nasal	deep			
Junco hyemalis		r. nasal	deep			
Melospiza fasciata		r. nasal	deep			
Passerella iliaca		r. nasal	deep			
Passer domesticus		r. nasal	deep			
Passerina cyanea		r. nasal	deep			
Tachycineta bicolor				r. nasal r. temporal	deep medium	
Hirundo urbica				r. nasal r. temporal	deep medium	Chievitz
Hirundo rustica				r. nasal r. temporal band-like	deep medium shallow	Chievitz
Vireo flavifrons		r. nasal	deep			
Dendroica palmarum		r. nasal	deep			
Sciurus aurocapillus		r. nasal	deep			
Geothlypis philadelphia		r. nasal	deep			
Acçentor modularis		nasal	deep			Chievitz
Sylvania mitrata		r. nasal	deep			
Sylvia hypolais		nasal	deep			Chievitz

	No Area Found	Simple		More Complex		First Observer
		Area	Fovea	Area	Fovea	
Sylvia schœnobænus		nasal	deep			Chievitz
Sylvia cinerea		nasal	deep			Chievitz
Sylvia hortensis		nasal	deep			Chievitz
Motacilla alba				nasal band-like	deep shallow	Chievitz
Motacilla flava				nasal band-like	deep shallow	Chievitz
Alauda arvensis		r. nasal	deep			Chievitz
Troglodytes parvulus		nasal	deep			Chievitz
Certhia familiaris		r. nasal	deep			
Parus cœruleus		nasal	deep			Chievitz
Parus major		nasal	deep			Chievitz
Parus atricapillus		r. nasal	deep			
Regulus satrapa		r. nasal	deep			
Regulus cristatus		nasal	deep			Chievitz
Saxicola rubethra				nasal band-like	deep shallow	Chievitz
Saxicola œnanthe				nasal band-like	deep shallow	Chievitz
Merula migratoria		r. nasal	deep			
Sialia sialis		r. nasal	deep			

REPTILIA						
Ophidia						
Boa constrictor		small				Hulke (14) [1867]
Natrix torquata		small				Hulke
Vipera communis		small				Hulke
Tropidontus natrix		round	o			Flesch [1876]
Coluber natrix		round				Flesch
Haldea striatula	(?)					
Eutainia sirtalis	(?)					
Liopeltis vernalis	(?)					
Lacertilia						
Phrynosoma cornutum		round band-like	deep o			
Chamæleo vulgaris		round	deep			Knox (11) [1823]
Lacerta scutata		round	deep			Knox
Lacerta vivipara		round	shallow			Chievitz
Lacerta viridis		round	shallow			Hulke (14) [1867]
"Spanish gecko"		round	(?)			Hulke
Iguana (tuberculata?)		small	(?)			Hulke
Superciliosa Calotes		round	medium			Knox
Testudinata						
Dermochelys coriacea		round				
Chelydra serpentina		round				
Chelopus insculptus		round				
Emys meleagris (?)		round				
Emys Europæa		round	o			Hulke
Testudo Græca		small				Hulke
Chelonia mydas		small				Hulke

	No Area Found	Simple		More Complex		First Observer
		Area	Fovea	Area	Fovea	
Crocodilia						
Crocodilus intermedius		band-like	shallow			Chievitz
Alligator Mississippensis		band-like	shallow			Chievitz
AMPHIBIA						
Urodela						
Salamandra atra		small				Hulke
Salamandra maculosa	o					Chievitz
Triton punctatus	o					Chievitz
Triton cristatus		small				Hulke
Diemyctylus viridescens	o					
Salientia						
Bufo viridis		long				Chievitz
Bufo calamita		long	o-shallow			Chievitz
Bufo vulgaris		round	o-shallow			Hulke
Bufo lentiginosus		band-like				
Hyla arborea		long	o			Chievitz
Rana temporaria		long				Hulke
Rana esculenta		band-like				Chievitz
Rana virescens		band-like				
Rana catesbiana		band-like				

PISCES						
Squali						
Squalus acanthias		oval				
Carcharhinus obscurus		(?)				
Raiæ						
Torpedo occidentalis		oval				
Dasyatis centrurus		oval				
Eventognathi						
Catostomus tères		(?)				
Notropis megalops		oval				
Isospondyli						
Brevoortia tyrannus		oval				
Haplomi						
Esox reticulatus		oval				
Apodes						
Anguilla anguilla		(?)				
Lophobranchii						
Syngnathus typhle		round	shallow			Krause (37) [1889]
Siphostoma fuscum		round	shallow			
Hippocampus (?)		round	shallow			Carrière (16) [1885]
Hemibranchii						
Gasterosteus aculeatus		(?)				
Percesoces						
Ammodytes tobianus amer.		(?)				

	No Area Found	Simple		More Complex		First Observer
		Area	Fovea	Area	Fovea	
Acanthopteri						
Seriola zonata		(?)				
Pomatomus saltatrix		oval				
Stromateus triacanthus		oval				
Perca flavescens		(?)				
Centropristis striatus		(?)				
Stenotomus chrysops		oval				
Cynoscion regalis		oval				
Ctenolabrus adspersus		oval				
Acanthocottus æneus		oval				
Prionotus carolinus		oval				
Batrachus tau		oval				
Pagellus centrodontus (?)		round	shallow			Gulliver (15) [1868]
Heterosomata						
Paralichthys dentatus		oval				
Pleuronectes platessa		round	shallow			Schiefferdecker (38) [1884]
Plectognathi						
Orbidus maculatus		(?)				
Pediculati						
Lorphius piscatorius		(?)				

The following tabulation, condensed from the foregoing, will show at a glance the prevalence of an area and fovea centralis in the different classes of vertebrates.

Number of Species		No Area Found	No Fovea Found	Area			Fovea		
				One Round	Two Round	Band-like	One Simple	Two Simple	Trough-like (?)
51	Mammals	10	38	31		8	18		
102	Birds	0	1	59	11	36	72	11	22
25	Reptiles	3 (?)	17	20		3	6		2
14	Amphibians	3	11	3		8	2		
30	Fishes	10	25	20			5		

Mammals.

Mammals as a class are characterized by the absence of a fovea, the primates being the only ones in which it has been found. As a rule an area is present, though in some cases even an area has not been demonstrated. H. Müller (13) says: "In mammals there is at least an area centralis present which approaches in structure the yellow spot, and is made known by a similar course of the blood-vessels as in man." If such is the case, I have failed in some instances to demonstrate the presence of such an area.

In some mammals the area is readily seen with the naked eye, but in the majority of those I have been able to examine such is not the case. In many instances vertical and horizontal sections have to be made and subjected to microscopical examination and measurement before a thickening or an arrangement of cells indicating an area is found. In some the very slight thickening is marked also by an increase in thickness of the tapetum.

I will now proceed to a more detailed description of the area and fovea in the mammals which I have studied. I shall not, however, enter into the histological arrangement of the cells.

Human and Gorilla.

The fovea and macula lutea are readily seen, located about 4 mm. toward the nasal side of the entrance of the optic nerve. The macula is rather sharply marked off from the surrounding retina, and is of small extent compared with other mammals. The blood-vessels in either of these cases were not injected, but they could be traced as far as represented in Pl. XXVII, Figs. 1, 2. Figs. 24, 25, Pl. XXVIII, represent horizontal sections through a child's eye and an adult's respectively. The foveola described by Dimmer (29) is much more noticeable in the adult (Fig. 25) than in the child's fovea. In the case of the gorilla, which was about nine hours *post mortem*, folds had formed about the fovea so that its appearance is not well represented in sections. Pl. XXVIII, Fig. 26, represents the horizontal section and Fig. 27 the vertical section through the center of the fovea.

Rabbit (Lepus sylvaticus).

The nerve entrance is readily seen above the center and a little toward the temporal side. From it two large bundles of nerve fibres branch out horizontally. In the injected specimen the blood-vessels are seen to lie in these bundles, and do not branch over the rest of the retina. The band-like area is seen to extend horizontally across the retina, immediately below the nerve entrance, and to gradually fade out just before reaching the ora serrata. It is from ½ to 1 mm. broad (Pl. XXVII, Fig. 13).

Rat (Mus rattus).

I have not succeeded in demonstrating the presence of a definite area in this animal.

Woodchuck (Arctomys monax).
Red Squirrel (Sciurus hudsonicus).
Fox Squirrel (Sciurus niger).
Chipmunk (Tamias striatus).

No area is visible to the naked eye, but in horizontal and vertical sections a slightly thicker oblong or oval area is dis-

cernible. This I have called the area centralis. It is situated near the center of the retina, but slightly above and toward the temporal side. The nerve entrance is very noticeable and of unusual shape. The nerve is flattened out fan-like just before piercing the sclerotic, so that the papilla is narrow and elongated. Pl. XXVII, Fig. 14, represents the entrance of nerve and the position of the area as nearly as I can ascertain it in Sciurus niger.

BAT (Vespertilio subulatus).

I have not been able with the material at hand to demonstrate an area.

SHEEP (Ovis avies).

Chievitz (31, *b*) has described this area as not visible to the naked eye, round, about 4 mm. in diameter, and located about 8 mm. toward the temporal side of the nerve entrance. I have examined more than 20 eyes, and in every case find a white, band-like region, about 1–2 mm. broad, extending horizontally across the retina, gradually becoming invisible to the naked eye just before reaching the ora serrata. It compares favorably in every respect with the area centralis of the cow. It lies above the nerve entrance, which is below the center and toward the temporal side. Pl. XXVII, Fig. 8, represents the position and extent of the area and its relation to the blood-vessels and nerve entrance in the left eye.

COW (Bos taurus domesticus).

A horizontal band-like area 1–2 mm. broad is present, having the same general relation to the nerve entrance and blood-vessels as found in the sheep (Pl. XXVII, Fig. 6).

PIG (Sus domesticus).

A band-like area about 1 mm. broad passes horizontally across the retina, and has the same relation to the blood-vessels and nerve as that described for the sheep and cow. The nerve entrance is nearer the center of the retina (Pl. XXVII, Fig. 9).

Horse (Equus caballus).

The band-like area is here very broad, 5–7 mm., and extends horizontally across the retina. The nerve entrance is below the center and slightly toward the temporal side. The blood-vessels are very meagre, and, according to Müller, extend over but a small portion of the retina, leaving the area centralis and the entire upper part of the retina free from blood-vessels. The blood-vessels are usually not visible unless they are injected (Pl. XXVII, Fig. 7).

Cat (Felis catus domesticus).

Chievitz (31, *c*) has described the area as round and not visible to the naked eye and located toward the temporal side of the nerve entrance. Ganser (19) has described it as round, and Krause (18) has stated that the cat possesses a fovea as well as an area. The retinal blood-vessels (Pl. XXVII, Fig. 4) would indicate as much as those of the sheep or cow the presence of a band-like area. Or the finer branches radiating toward a common point on the temporal side of the nerve entrance would suggest a round area similarly located as that described by Chievitz. In most cases a region similar to that indicated by the dotted lines and having the same macroscopical appearance as an area is observed. This appearance may be due to the tapetum lying behind. The lower margin of this area-like region corresponds very closely with that of the tapetum. In sections I have not found a well-defined round area, but a general thickening over the greater part of the region indicated.

Skunk (Mephitis mephitica).
Mink (Putorius vison).

No area is visible to the naked eye, but in horizontal and vertical sections an oblong or oval thickening is found located a little above and on the temporal side of the nerve entrance, which in these animals is central.

Fox (Vulpes vulpes).

A horizontal, band-like region extending across the retina just above the nerve entrance may be seen with the naked eye

(Pl. XXVII, Fig. 5). In the representation the retinal vessels were not injected, and the smaller branches could not be accurately made out. In cross sections only a slight thickening of the retina is noticed, and the lower edge of the indicated region corresponds with the lower margin of the tapetum.

Dog (Canis familiaris).

The retinal blood-vessels (Pl. XXVII, Fig. 3) indicate the presence of a band-like area. Again the finer and more numerous branches radiating toward a common spot on the temporal side of the nerve entrance points to the presence of a round area. Neither are visible to the naked eye, but Chievitz (31, *b*) has described the presence of a round area in this latter position. I have not succeeded in demonstrating it.

Birds.

Birds are characterized by the presence of a fovea, although a few cases are very doubtful (hen and guinea hen). Chievitz says (31, *c*) that at least a round area is always present which regularly possesses a fovea, sometimes very clearly seen, and in other cases so shallow as to be very doubtful (duck and hen).

Where but a single fovea is present the position and form are so similar, as shown in the tabulation, that a large number may be described together. As a rule it is situated about the center of the retina, a short distance above and toward the nasal side of the optic nerve entrance. The nerve entrance is always more or less obscured from view by the pecten, which extends obliquely from the point of entrance downward and forward, so that a line joining the fovea and nerve entrance forms about a right angle with the pecten (Pl. XXVII, Figs. 17, 23). The fovea, with but few exceptions which will be described separately, is surrounded by a simple round area more or less sharply marked off from the surrounding retina. The fovea varies considerably in depth. In the tabulation I have classified them as deep, medium, and shallow.

Most birds possess a deep and well-defined fovea, as seen in the following:

Robin (Merula migratoria, Pl. XXVII, Fig. 17, and Pl. XXVIII, Fig. 28).
Blue-Bird (Sialia sialis, Pl. XXVIII, Fig. 29).
Kinglet (Regulus satrapa, Pl. XXVIII, Fig. 30).
Snow-Bird (Junco hyemalis, Pl. XXVIII, Fig. 31).
Crow (Corvus americanus, Pl. XXIX, Fig. 52).
Blue Jay (Cyanocitta cristata, Pl. XXIX, Fig. 53).
Night Heron (Nycticorax nycticorax, Pl. XXVII, Fig. 23).

A number of birds possess a medium fovea, as seen in the pigeon (Columba livia domestica, Pl. XXVIII, Figs. 37, 38). It is readily observed surrounded by a well-defined area. It varies somewhat in depth in the same species, as is shown in this case. Fig. 37 represents a medium fovea, while Fig. 38 would be classed as shallow.

Most of the Gallinæ which I have examined possess medium to very shallow fovea. The quail (Colinus virginianus) and the partridge (Bonasa umbellus) each possess a medium fovea, while in the turkey (Meleagris gallopavo, Pl. XXVIII, Fig. 35) and the guinea hen (Numida pucherani, Pl. XXVIII, Fig. 36) it is shallow. In the last case the depression is so slight as to scarcely deserve the name of fovea. Chievitz mentions an area nasalis and a questionable fovea in the hen (Gallus domesticus). I have succeeded in finding only a very slight thickening.

Screech Owl (Megascops asio).
Barred Owl (Syrnium nebulosum).

These owls possess a single deep fovea surrounded by a sharply defined round area which differs from those just described only in position. It is located on the temporal side and above the nerve entrance in such a position as to function in binocular vision. The nerve entrance is similar in position to that of other birds, but the pecten is much smaller in proportion to the size of the eye (Pl. XXIX, Fig. 55, and Pl. XXVII, Fig. 10).

Goose (Anser cinereus domesticus).

The goose possesses a shallow fovea nasalis surrounded by a round area situated on a band-like area extending horizontally through the retina. The fovea and round area are easily observed with the naked eye, but the band-like area is much

less distinct. Vertical sections across this area show only a slight increase in thickness, both on the nasal and temporal side of the fovea (Pl. XXVIII, Figs. 32–34).

TAME DUCK (Anas boschas domesticus).
SURF DUCK (Oidemia deglandi).

Similar relations exist here as in the goose. The fovea is quite shallow, and is surrounded by a distinct round area which is situated on a band-like horizontal area (Pl. XXVIII, Fig. 39).

RING-NECK PLOVER (Ægialitis semipalmata).

A very distinct band-like area is seen passing obliquely through the retina. A dark line, resembling a trough-like fovea, extends almost the full length through the center of this area. Cross sections reveal, however, no trough-like fovea. The single fovea nasalis, surrounded by a sharply bounded round area, is observed located about the middle of the band-like area. It is of medium depth and readily seen by the naked eye (Pl. XXVII, Fig. 20, and Pl. XXIX, Fig. 47).

SPARROW HAWK (Falco sparverius).

A fovea nasalis and a fovea temporalis, each surrounded by a sharp round area connected by a short band-like area, are easily observed. The fovea nasalis is very deep and sharp and is situated about the center of the retina. The fovea temporalis, somewhat shallower, is situated near the ora serrata about the same distance from the nerve entrance as the fovea nasalis, but in a lower plane. The area temporalis is likewise smaller than the area nasalis. The band-like area is not sharply bounded, is of slight thickness, and extends only between the two round areas. The fovea temporalis is similar in position to that of the owl, and the fovea nasalis to that of the crow, robin, etc. (Pl. XXVII, Fig. 19, and Pl. XXIX, Figs. 48, 49).

RED-TAILED BUZZARD (Buteo borealis).

Almost the same conditions exist as found in the sparrow hawk, excepting the two foveæ are relatively nearer together (Pl. XXVII, Fig. 11, and Pl. XXIX, Figs. 50, 51).

Kingfisher (Ceryle alcyon).

The same conditions exist as are found in the hawk (Pl. XXIX, Figs. 44, 45).

White-Bellied Swallow (Tachycineta bicolor).

A fovea nasalis and a fovea temporalis are easily seen, each surrounded by a round area situated on a band-like area extending obliquely across the retina. The positions of the foveæ are very similar to those described in the hawks. The fovea and area temporalis are likewise smaller, and are situated nearer the ora serrata than the fovea and area nasalis in the hawks. The area and fovea nasalis are shown in Pl. XXIX, Fig. 46.

Common Tern (Sterna hirundo).

In this case both nasal and temporal foveæ surrounded by round areas are present, and in addition a band-like area. The area nasalis is located on the band-like area about the center of the retina, but the area temporalis is above the band-like area, and apparently in no way connected with it. A dark line, resembling a trough-like depression, extends through the center of the band-like area, through the fovea nasalis, and terminates near the entrance of the optic nerve (Pl. XXVII, Fig. 12). A cross section of this area, given in Pl. XXIX, Figs. 40, 42, fails to demonstrate such a depression. The temporal end of the band-like area widens and soon becomes indistinct. The fovea temporalis is very shallow and might be overlooked. It is located near the ora serrata a little above the median horizontal plane. The fovea nasalis is deep and easily observed (Pl. XXIX, Figs. 41, 43).

Reptiles.

In the tabulation twenty-five species are mentioned. All but three are described as having an area, and these three are questionable. Eight of the number possess a well-defined fovea, while two are doubtful.

In snakes an area seems to be the rule. In the three species I have examined, the retina was not sufficiently well

preserved to make certain the presence of an area. It is only visible in sections.

In the lizard an area has been described in every case, and a fovea in all but two, which are doubtful. The only lizard which I have examined, the horned toad (Phrynosoma cornutum), possesses a deep and sharp fovea, situated on a broad band-like area. The fovea is situated about the center of the retina, just above the entrance of the optic nerve, which is marked by a slender conical pecten. The band-like area is broadest in the region of the fovea, and extends horizontally across the retina. A dark line extends about 1 mm. to either side from the fovea and gives the appearance of a trough-like fovea, as seen in the tern, but cross sections reveal no depression. The band-like area gradually becomes indistinct some distance from the ora serrata (Pl. XXVII, Fig. 15, and Pl. XXX, Figs. 56, 57).

In the turtles only an area has been found which is oval or round in shape, and lies about the center of the retina, just above the nerve entrance. It is not visible to the naked eye, and in sections is noticed rather as a closer arrangement of the cells than as a thickening. Pl. XXX, Fig. 61, represents a section through the area of Chelydra serpentina. A representation of the entire retinal section would be necessary to show any difference in thickness. In an injected specimen of Chelopus insculptus, a short and seemingly rudimentary blood-vessel was noticed (Pl. XXVII, Fig. 16) which seemed to be an approach to a retinal circulation. In the other eye it was not so long but similarly located.

In the crocodiles Chievitz has described and pictured a band-like area and shallow trough-like fovea which extend horizontally through the entire retina. I have not been able to examine any species of this order.

Amphibians.

The presence of an area and absence of a fovea seems to be the rule. Hulke and Chievitz, however, have described a shallow fovea in Bufo vulgaris and Bufo calamitia, though in some cases it is wanting. I have found a band-like area in Bufo lentiginosus, Rana virescens and catesbiana. It is not visible

to the naked eye and is demonstrated only in vertical sections by a slight and gradual increase in thickness, principally in the inner nuclear layer and in the closer arrangement of the nerve cells. The position of the area is outlined in Pl. XXVII, Fig. 18, as found in Rana catesbiana. Pl. XXX, Fig. 62, represents the vertical section through the area.

Fishes.

Fishes seem to be characterized, as a rule, by the absence of both a fovea and a well-defined area. Nothing is visible to the naked eye excepting in a few cases, which will receive special mention. If sections of the eye, however, are subject to microscopical measurement, an oblong or oval region, slightly thicker than the rest of the retina, is found located on the temporal side and a little above the center. In fact, the whole upper half of the retina is somewhat thicker than the lower half in all fishes which I have examined. That region indicated above, however, is the thickest, and I have designated it the area centralis. It also corresponds in position to that of the fovea when a fovea is present. Some of the material at hand was not sufficient to demonstrate clearly the presence of such an area. Such cases I have indicated as doubtful. Pl. XXX, Fig. 63, represents a section through the area of the flounder (Paralichthys dentatus), but no increase in thickness is visible in so small a portion of the retina.

Krause (37) has described the presence of a round area and shallow fovea in Syngnathus typhle, and Carrière (16) has described and pictured a similar area and fovea in Hippocampus. Gulliver (15) has described a round area and shallow fovea in Pagellus centrodonpus (?). Schiefferdecker (38) has described a similar area and fovea in Pleuromectes platessa. I have not been able to procure any of these species, but have found an area and fovea in another species.

Pipefish (Siphostoma fuscum).

The eye of this fish being so small, I have not attempted a macroscopical examination. The area and fovea are, however,

probably visible to the naked eye. In horizontal sections the area and fovea may be readily seen. The fovea is broad and shallow when compared with that of most birds and some reptiles. It is located on the temporal side, about midway between the nerve entrance and the ora serrata, and a little above. A horizontal section through the nerve passes through the area below the fovea, as shown in Pl. XXX, Fig. 59 (1). A section through the fovea is shown in Fig. 58 (1).

Physiological.

In order to make a physiological comparison of the areas of acute vision in the different vertebrates, the exact function of the different elements of the retina must be known. Most physiologists agree on the functions of all the elements excepting the rods and cones. All are agreed, however, that the rods and cones are the elements which give the sensation of sight, but just the function of each is very obscure. The source of information regarding the functions of the rods and cones has necessarily been confined to man. When this has been finally settled, a more accurate comparison of the powers of sight in the different vertebrates can be made.

A great many theories have been advanced regarding the functions of the rods and cones, and as these theories cannot be fully verified or tested by physiological experiments, they will have to be accepted as such.

What the changes are which take place in the retina during an act of sight had long been a mystery till the visual purple was discovered in the rod and cone layer by Boll. This, however, sufficed for only a short time, as it was soon found that the cones possessed no visual purple, or at least none could be demonstrated in them. Since the cones are the only sensitive elements in the fovea, some other photo-chemical substance must be present in them. The theories of Young-Helmholtz, Herring, Mrs. Franklin, etc., agree generally in the functions of the rods and cones, but differ in the photo-chemical substance and its change in an act of sight. Since the theories of Young-Helmholtz and Herring do not ascribe different func-

tions to the rods and cones, I shall not refer to them further. Mrs. Franklin (39) bases her theory on carefully conducted experiments testing the sensitiveness of different regions of the retina to various colors and intensities of light. She assumes the presence of two kinds of molecules in the photochemical substance of the retina: (1) gray molecules which give rise to the sensation of gray; (2) color molecules which have been differentiated from the gray, and whose atoms of the external layer are arranged in three directions at right angles to each other. These give the sensation of color. She would thus attribute to the rods the perception of uncolored light, for she says (40, *a*): "In the very eccentric part of the retina the differentiation of the color molecule out of the gray molecule has not taken place; these parts of the retina are chiefly useful to us in warning us of danger from moving insects and other enemies, and for this the power to detect differences of brightness is sufficient." Again (41): "*Only the cones* are sensitive to variations of color; they must be extremely sensitive to variations of intensity in white light as well, — otherwise the fovea would not be the place with which we make out the minutest variations of line and shade in an intricate drawing. If the *cones only* give color, they do not give *color only*." Her experiments, as well as those of König (44), further show that the fovea is blind to blue, and is not able to perceive other colors when the illumination is faint, seeing them only as "different intensities of gray" (40, *b*). In color-blind people she finds that they are blind in the center of the fovea, but have come to use a small spot on the edge of the fovea as the point of acute vision (42). The maximum sensitiveness of the retina to faint impression is found to be about 25° from the fovea where it is four times as sensitive, and at 50° it is still twice as sensitive as the fovea. These gray and color molecules are, of course, only theoretical and cannot be demonstrated. The gray molecules, without doubt, correspond to the visual purple of other writers, which is found only in the rods. The results of the various experiments on the sensitiveness of the retina to different colors correspond closely with the arrangement of the rods and cones. In the center of the

fovea, where only cones exist, colors are most easily perceived, while in the periphery, where there are few cones, it is difficult to distinguish them.

M. H. Parinaud (43) has found by experiment on the excised retina that the visual purple (hence the rods) cannot be demonstrated nearer the center of the fovea than two millimeters. From this place the rods are found to increase in number toward the periphery and the cones to decrease.

Again, the retina being four times as sensitive to faint impressions 25° from the fovea as at the fovea, and since at this distance the rods are far more numerous than the cones, we can consider the functions of the rods fairly well determined to be the perception of diffuse and gray lights.

To sum up: (1) the rods and cones are the sensitive elements of sight; (2) the rods give us the sensation of gray, while the cones give us the sensation of color and gray; (3) the rods are more sensitive to faint impressions than the cones; (4) the elements of the other layers form the connection with the optic nerve.

With this in view concerning the functions of the retinal elements in man, and supposing the functions to be the same in the other vertebrates, a physiological comparison may be attempted.

Quite a difference is noticed in the relative thickness of the layers of the retina of the different vertebrates. This is shown diagrammatically in Pl. XXVII, Fig. 22. The layers which exhibit the greatest difference are the inner and outer nuclear layers and the rod and cone layer. In mammals the outer nuclear layer is much thicker than the inner, while in birds, reptiles, amphibians, and fishes the reverse is true.

The layer which shows the greatest diversity, however, is that of the rods and cones. A great difference exists in their size, length, shape, and relative number. Fishes possess the longest rods, while amphibians have not only long rods, but also the thickest found in the vertebrates. The rods of mammals are long but very slender, while in birds they are comparatively short and thick. The cones are the longest and most slender in some of the reptiles (chameleon), and of greatest

diameter in the mammals. They are about the same length in mammals, birds, and amphibians, while in fishes they are shorter. In birds the diameter of the cones approaches very closely to that found in the reptiles. The following tabulation of measurements compiled from Müller's descriptions (20) of the rods and cones of different animals will make clear these relations. These measurements are in millimeters.

	Rods		Cones	
	Diameter	Length	Diameter	Length
Human	.0015–.0018	.04–.06	.004–.006	.032–.036
Pigeon	.0026–.0033	.02–.028	.001–.005	.025–.03
Chameleon			.001–.0013	.06–.08
Frog	.006–.007	.04–.06	.005	.02–.028
Perch	.0026	.04–.05		.008–.012

The diameter of the rods and cones is of great importance when the sensitiveness of the retina of different animals is considered. Since these sensitive elements always lie as closely together as possible, the animals in which their diameter is small would have more per given area, hence a more sensitive retina.

Another important difference is the relative number of rods and cones. In mammals and amphibians the rods far surpass the cones in number. In birds the reverse is true, while in reptiles few or no rods are found. In fishes the rods and cones are more equally divided. A few exceptions to this are of great importance in substantiating the theories of the functions of the rods and cones. It has been stated (45) that in the bat and mole there are no cones in the yellow spot and in the rabbit only a few. The same is true of other nocturnal mammals which I have examined. I have not been able to demonstrate the presence of cones in the mink, skunk, or rat, while they are present in the squirrels. In the night birds and in the eel very few or no cones have been demonstrated. This accords completely with the theory that the rods function in

the perception of uncolored and diffuse light. Since all colors appear as gray by diffuse light, even though perceived by the cones, and since the rods are more sensitive to faint impressions than the cones, the presence of rods and almost complete absence of cones in night animals is no more than can be expected. Again, since the perception of color is one of the important functions in day animals, and as this is done only by the cones, the relatively greater number of cones in these animals is readily accounted for.

Acute vision, however, seems to depend on the presence of a fovea. In man the power to see distinctly grows rapidly less from the fovea to the ora serrata. The macula, it is true, sees objects more distinctly than the peripheral parts of the retina, but even this functions with the peripheral part more as a sentinel for moving objects than as a point of acute vision. It is true that all animals are attracted more quickly by moving objects than by stationary ones, and it is especially true in those animals whose retinal development has not proceeded beyond the differentiation of an area. The power of quiet and close discrimination of objects at rest seems to be present only with those animals which possess a fovea.

Fishes as a rule depend upon sight for their food, excepting such as the shark, which depends almost wholly on its smell. This class of vertebrates does not, however, usually possess a fovea. How distinctly they see we cannot say, but we know that the trout quickly takes the fly when thrown on the water, or the pickerel the whirling spoon as it is drawn before it. They see the objects while in motion, and are apparently unable to distinguish them from the real article of food. An experience in fishing confirms the fact that a pickerel will not bite at a motionless spoon-hook. The retina of these fish has simply a thickening or area at the axis of vision.

A somewhat similar experiment can be tried with the frog or toad. If one attaches a bit of red flannel, a green leaf, or any other small object to a thread and dangles it before a hungry frog, he will quickly jump for it. A toad may be fed on meat in a similar way, but in no case will the meat be taken unless it is in motion. Neither do these animals show any marked power

of discrimination by sight. They will jump at any small moving object, and are apparently not able to distinguish till they have it in their mouths whether it is an article of food or a pebble. Investigations again show the presence of an area and absence of a fovea.

In some of the reptiles, however, a marked difference in power of discrimination by sight is noticed. Experiments were made wholly on a small lizard (horned toad). If a dead fly were put before him when he was hungry he would eye it closely for a brief time, then quickly take it. His aim was always certain, never missing his mark, while that of the ordinary toad was more at random, throwing out her tongue indiscriminately at moving objects. It is true the lizard was attracted more by a live and moving fly than by a dead, motionless one, but he also had the power of perceiving things at rest. This little creature possessed a sharp and well-defined fovea.

In general, birds' eyes are almost as perfect as man's, and likewise the optic lobes are even greater in proportion to the size of the body than that of man. It is true that the bird often catches flies as they buzz about, but it also inspects each leaf carefully above and below for a worm or bug which may be there in hiding, and which it seldom fails to recognize. The hawk as it soars high in the heavens sees the snake, rat, or mouse in the grass, and is frequently seen to dart and secure its prey. Very acute sight is present in all birds and especially in birds of prey.

A great difference exists in the power of sight in mammals. The primates possess the power of most acute vision. Many of the mammals depend on smell and hearing more than on sight. The dog picks his master out of the crowd by smell; so does the sheep her lamb. Sight in these cases being only partial recognition, as they are not sure until they have confirmed their sight by the sense of smell. The same is true of the cow, for she must smell of the strange cow when introduced into the herd. The horse is cured of his fright by smelling of the object which caused it. In all these cases we have a motion of the ears, showing that the animal is not only using sight and

smell, but also hearing. Mammals in general do not see a man if he remains quiet, but the crow easily recognizes him, and can distinguish his stick from a gun. The dog looks into your face, but you cannot tell whether he is looking into your eyes or at your mouth. He has an indefinite gaze, and, like most mammals, is not satisfied with the sense of sight alone, but must confirm and improve by the sense of smell and hearing.

In the present study it is impossible to make a more definite comparison of the powers of vision in the different vertebrates. Many years of careful observation of the visual habits and related histological structure of each animal will be necessary. But so far as experiments have gone, the power of quiet and close discrimination of an object at rest seems to be present only in those animals whose development of the retina has proceeded a stage farther above that of the simple area — to the fovea centralis.

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EXPLANATION OF PLATE XXVII.

FIG. 1. Human, left eye, $\frac{1}{1}$, showing nerve entrance (*N*), blood-vessels, and macula and fovea (*A F*).

FIG. 2. Gorilla, left eye, $\frac{1}{1}$. *N*, nerve entrance; *A F*, area and fovea.

FIG. 3. Dog (Canis familiaris), left eye, $\frac{1}{1}$. Shows nerve entrance (*N*) and blood-vessels which were injected.

FIG. 4. Cat (Felis catus domesticus), left eye, $\frac{1}{1}$. *N*, nerve entrance; *Ab*, white, band-like region, which appears as an area. The blood-vessels were injected.

FIG. 5. Fox (Vulpes vulpes), left eye, $\frac{1}{1}$. *N*, nerve entrance; *Ab*, band-like area. Blood-vessels were not injected.

FIG. 6. Cow (Bos taurus domesticus), left eye, $\frac{1}{1}$. *N*, nerve entrance; *Ab*, band-like area.

FIG. 7. Horse (Equus caballus), left eye, $\frac{1}{1}$. *N*, nerve entrance; *Ab*, band-like area.

FIG. 8. Sheep (Ovis avies), left eye, $\frac{1}{1}$. *N*, nerve entrance; *Ab*, band-like area. Blood-vessels were injected.

FIG. 9. Pig (Sus domesticus), left eye, $\frac{1}{1}$. *N*, nerve entrance; *Ab*, band-like area.

FIG. 10. Barred Owl (Syrnium nebulosum), left eye, $\frac{1}{1}$. *N*, nerve entrance; *P*, pecten; *A F*, area and fovea.

FIG. 11. Red-Tailed Buzzard (Buteo borealis), left eye, $\frac{1}{1}$. *N*, nerve entrance; *P*, pecten; *Ft*, *At*, fovea and area temporalis; *Fn*, *An*, fovea and area nasalis; *Ab*, band-like area.

FIG. 12. Tern (Sterna hirundo), left eye, $\frac{1}{1}$. *N*, nerve entrance; *P*, pecten; *Ft*, *At*, fovea and area temporalis; *Fn*, *An*, fovea and area nasalis; *Ab*, band-like area. A dark line extending along the band-like area corresponds to Chievitz's trough-like fovea. In cross sections no such fovea is found.

FIG. 13. Rabbit (Lepus sylvaticus), left eye, $\frac{1}{1}$. *N*, nerve entrance; *Ab*, band-like area. The blood-vessels were injected.

FIG. 14. Fox Squirrel (Sciurus niger), left eye, $\frac{1}{1}$. *N*, nerve entrance; *Ab*, area not visible to the naked eye.

FIG. 15. Horned Toad (Phrynosoma cornutum), left eye, $\frac{1}{1}$. *N*, nerve entrance; *P*, conical pecten; *F*, fovea; *Ab*, band-like area.

FIG. 16. Turtle (Chelopus insculptus), left eye, $\frac{1}{1}$. *N*, nerve entrance; *A*, area, not visible to the naked eye; *Bv*, an apparent rudimentary blood-vessel which was injected.

FIG. 17. Robin (Merula migratoria), left eye, $\frac{1}{1}$. *N*, nerve entrance; *P*, pecten; *A F*, area and fovea.

FIG. 18. Frog (Rana catesbiana), left eye, $\frac{1}{1}$. *N*, nerve entrance; *Ab*, band-like area, not visible to the naked eye.

FIG. 19. Sparrow Hawk (Falco sparverius), left eye, $\frac{1}{1}$. *N*, nerve entrance; *P*, pecten; *An*, *Fn*, area and fovea nasalis; *At*, *Ft*, area and fovea temporalis; *Ab*, band-like area.

FIG. 20. Ring-Neck Plover (Ægialitis semipalmata), left eye, $\frac{1}{1}$. *N*, nerve entrance; *P*, pecten; *A F*, area and fovea; *Ab*, band-like area.

FIG. 21. Chicken (Gallus domesticus), left eye, $\frac{1}{1}$. *N*, nerve entrance; *P*, pecten.

FIG. 22. Diagrammatic representation of the comparative thickness of the layers of the retina in the different vertebrates. Measurements were taken of the retina at corresponding positions and magnified 130 diameters. 1. Nerve-fibre layer; 2. Nerve-cell layer; 3. Inner molecular layer; 4. Inner nuclear layer; 5. Outer molecular layer; 6. Outer nuclear layer; 7. Rod and cone layer; 8. Pigment layer. The last two layers generally overlap. I. Human. II. Cat (Felis catus domesticus). III. Blue Jay (Cyanocitta cristata). IV. Snake (Eutainia sirtalis). V. Frog (Rana catesbiana). VI. Pickerel (Esox reticulatus).

FIG. 23. Black-Crowned Night Heron (Nycticorax nycticorax), left eye, $\frac{1}{1}$. *N*, nerve entrance; *P*, pecten; *AF*, area and fovea.

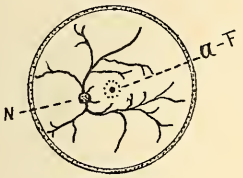

Fig. 1.

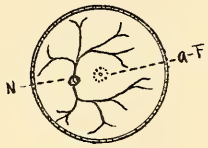

Fig. 2.

Fig. 3.

Fig. 4.

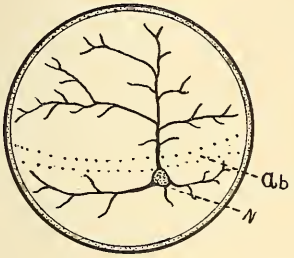

Fig. 6.

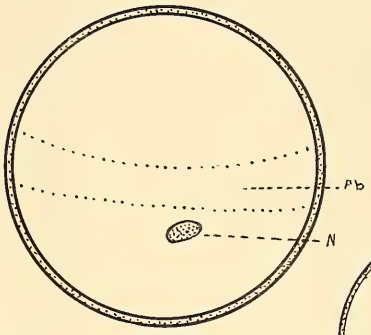

Fig. 7.

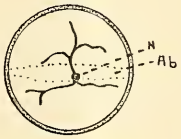

Fig. 5.

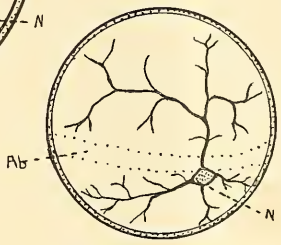

Fig. 8.

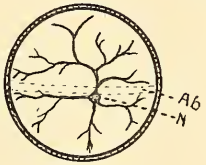

Fig. 9.

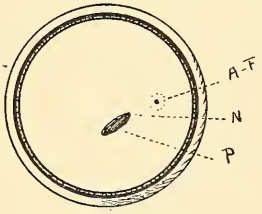

Fig. 10.

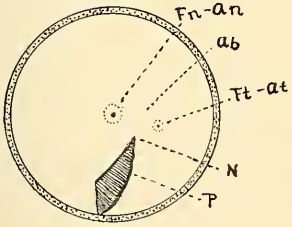

Fig. 11.

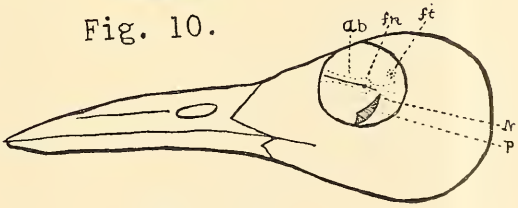

Fig. 12.

EXPLANATION OF PLATE XXVIII.

FIG. 24. Human, age 4. Horizontal section through the center of fovea of the right eye. The eye was enucleated during life and immersed at once in the hardening fluid. Section 36μ thick. × 32.3.

FIG. 25. Human, adult. Horizontal section of right eye through center of fovea. Eye was enucleated during life and subjected at once to the hardening fluids. Section 36μ thick. × 32.3.

FIG. 26. Gorilla. Horizontal section of right eye through center of fovea. The eye was about nine hours *post mortem* and the apparent depth of fovea is due to the folds of the retina in the macula. Section 36μ thick. × 32.3.

FIG. 27. Gorilla. Vertical section through the center of the fovea and the folds of the macula, showing the folds as they appear due to *post mortem* changes. Section 36μ thick. × 32.3.

FIG. 28. Robin (Merula migratoria). Horizontal section through center of the fovea of right eye. Eye was subjected to hardening fluids immediately after death. Section 24μ thick. × 32.3.

FIG. 29. Blue-Bird (Sialia sialis). Horizontal section through center of the fovea of the left eye. Subjected to hardening fluids immediately after death. Section 18μ thick. × 32.3.

FIG. 30. Kinglet (Regulus satrapa). Horizontal section through center of the fovea of the right eye. The head was subjected at once after death to hardening fluids and sections afterwards made through whole head with eyes *in situ*. This section includes not only the bottom of the fovea, but also some of the cells of the area beyond. Section 24μ thick. × 32.3.

FIG. 31. Snow-Bird (Junco hyemalis). Horizontal section through center of the fovea of right eye. This eye was hardened by the injection method. The retina in the region of the fovea floated off from the choroid. Section 36μ thick. × 32.3.

FIG. 32. Goose (Anser cinereus domesticus). Vertical section of right eye. Across band-like area on the nasal side of the fovea about midway to the ora serrata. The arrow points to the center of the area. Eye was subjected to hardening fluids immediately after death. Section 36μ thick. × 32.3.

FIG. 33. Goose. Vertical section through the center of the fovea of right eye. Section 36μ thick. × 32.3.

FIG. 34. Goose. Vertical section of right eye across band-like area on the temporal side of fovea about midway to ora serrata. The arrow points to the center of the area. A fold in the section partly obscures the area. Section 36μ thick. × 32.3.

FIG. 35. Turkey (Meleagris gallopavo). Horizontal section through center of the fovea of right eye. Eye was immersed at once after death in hardening fluid. Section 36μ thick. × 32.3.

FIG. 36. Guinea Hen (Numida pucherani). Horizontal section through center of area of right eye. The eye was subjected to hardening fluids at once after death. The arrow points to the center of the area where a very slight pitting may be seen, which may possibly be called a fovea. Section 24μ thick. × 32.3.

FIG. 37. Pigeon (Columba livia domestica). Adult. Horizontal section through center of the fovea of right eye. Section 18μ thick. × 32.3.

FIG. 38. Pigeon. Horizontal section through center of fovea of right eye. Section 24μ thick. × 32.3.

FIG. 39. Surf Duck (Oidema deglandi). Horizontal section through center of the fovea of left eye. Eye about three hours *post mortem*. Section 30μ thick. × 32.3.

Fig. 24.

Fig. 25.

Fig. 26.

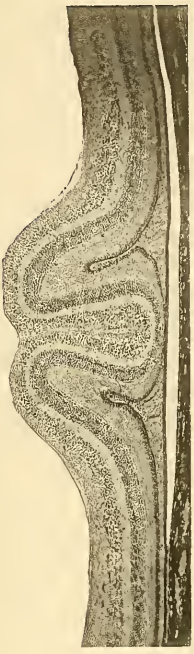

Fig. 27.

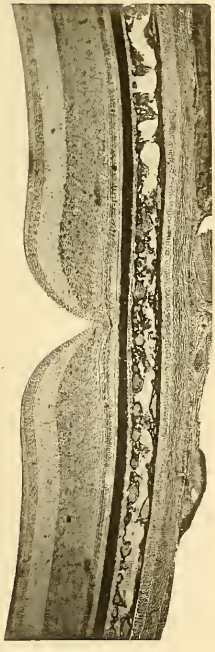

Fig. 28.

Fig. 29.

Fig. 30.

Fig. 31.

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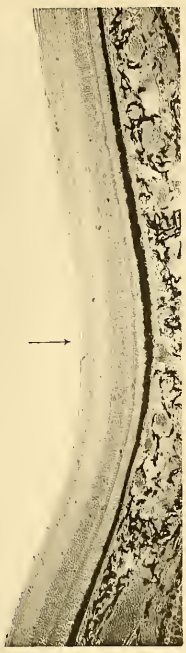

Fig. 32.

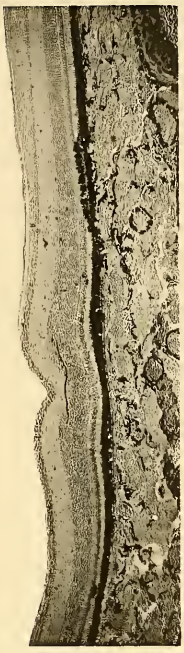

Fig. 33.

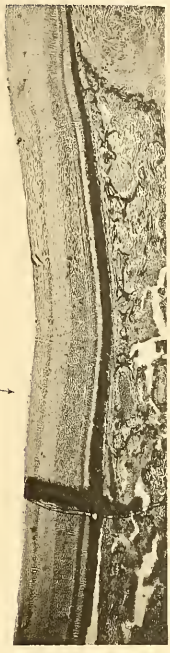

Fig. 34.

Fig. 35.

Fig. 36.

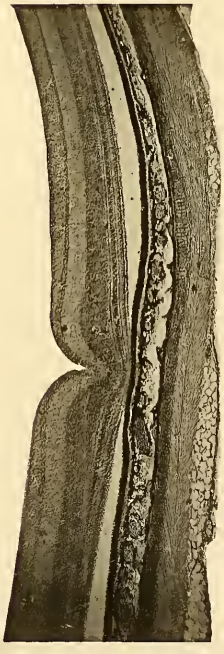

Fig. 37.

Fig. 38.

Fig. 39.

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EXPLANATION OF PLATE XXIX.

FIG. 40. Tern (Sterna hirundo). Vertical section across band-like area (*a*) on the nasalis side of the fovea nasalis. Section 36μ thick. × 32.3.

FIG. 41. Tern (Sterna hirundo). Horizontal section through center of fovea nasalis. Section 30μ thick. × 32.3.

FIG. 42. Tern (Sterna hirundo). Vertical section across band-like area (*a*) about midway between fovea nasalis and temporalis. Section 36μ thick. × 32.3.

FIG. 43. Tern (Sterna hirundo). Horizontal section through center of fovea temporalis. Section 30μ thick. × 32.3.

FIG. 44. Kingfisher (Ceryle alcyon). Horizontal section through center of fovea nasalis. Section 30μ thick. × 32.3.

FIG. 45. Kingfisher (Ceryle alcyon). Horizontal section through center of fovea temporalis. Section 30μ thick. 32.3.

FIG. 46. White-Bellied Swallow (Tachycineta bicolor). Horizontal section through center of fovea nasalis. Section 24μ thick. × 32.3.

FIG. 47. Ring-Neck Plover (Ægialitis semipalmata). Horizontal section through fovea of left eye. Section 18μ thick. × 32.3.

FIGS. 48, 49. Sparrow Hawk (Falco sparverius). Section passed through each fovea and center of pupil. Fig. 48, fovea nasalis and Fig. 49, fovea temporalis. Section 42μ thick. × 32.3.

FIGS. 50, 51. Red-Tailed Buzzard (Buteo borealis). Section passed in plane of both foveæ and center of pupil. Fig. 50, fovea nasalis and Fig. 51, fovea temporalis. Section 42μ thick. × 32.3.

FIG. 52. Crow (Corvus americanus). Horizontal section through center of fovea. Section 36μ thick. × 32.3.

FIG. 53. Blue Jay (Cyanocitta cristata). Horizontal section through center of the fovea. Section 48μ thick. × 32.3.

FIG. 54. Shorepeep (Ereunetes pusillus). Horizontal section through center of fovea of left eye. Shows some cells of the area lying beyond the center of the fovea. Section 24μ thick. × 32.3.

FIG. 55. Barred Owl (Syrnium nebulosum). Horizontal section of right eye through center of fovea (fovea temporalis). Section 48μ thick. × 32.3.

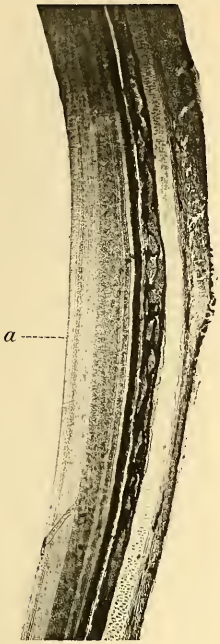

Fig. 40.

Fig. 41.

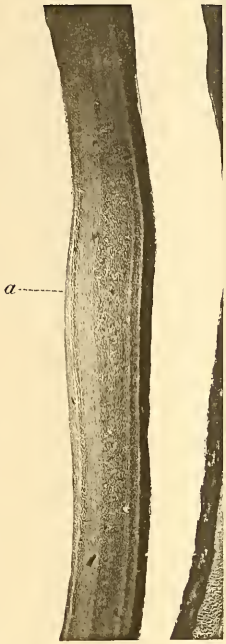

Fig. 42.

Fig. 43.

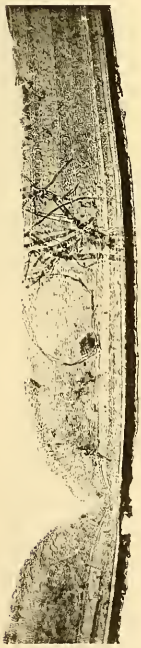

Fig. 44.

Fig. 45.

Fig. 46.

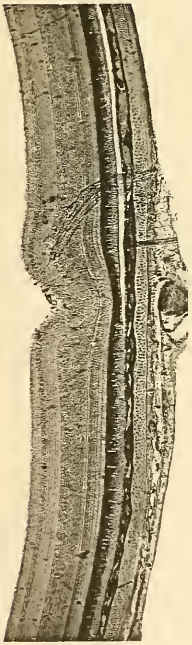

Fig. 47.

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Fig. 48.

Fig. 49.

Fig. 50.

Fig. 51.

Fig. 52.

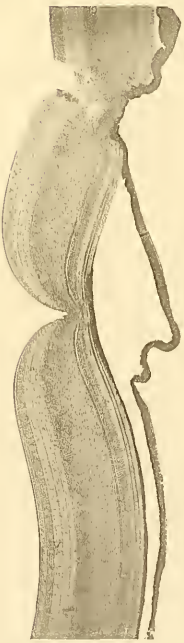

Fig. 53.

Fig. 54.

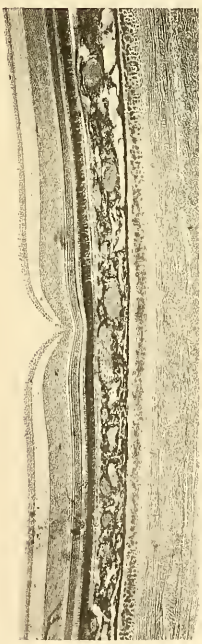

Fig. 55.

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EXPLANATION OF PLATE XXX.

FIGS. 56, 57. Horned Toad (Phrynosoma cornutum). Fig. 56, vertical section and Fig. 57, horizontal section through the center of each fovea. Fig. 56 shows also the width of the band-like area. Sections 18μ thick. × 32.3.

FIG. 58. Pipefish (Siphostoma fuscum). Horizontal section through center of fovea (1) of left eye. 2 indicates the position of nerve entrance. Section 18μ thick. × 32.3.

FIG. 59. Pipefish (Siphostoma fuscum). Section in a lower plane, showing entrance of nerve (2) and the area (1). Section 18μ thick. × 32.3.

FIG. 60. Chipmunk (Tamias striatus). Vertical section across area (*a*). Section 24μ thick. × 32.3.

FIG. 61. Turtle (Chelydra serpentina). Horizontal section through band-like area. Section 18μ thick. × 32.3.

FIG. 62. Frog (Rana catesbiana). Vertical section across band-like area (*a*). Section 24μ thick. × 32.3.

FIG. 63. Flounder (Paralichthys dentatus). Vertical section, showing comparative thickness of different layers of the retina. Section 30μ thick. × 32.3.

Fig. 56.

Fig. 57.

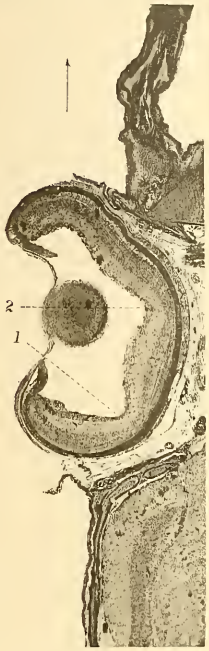

Fig. 58.

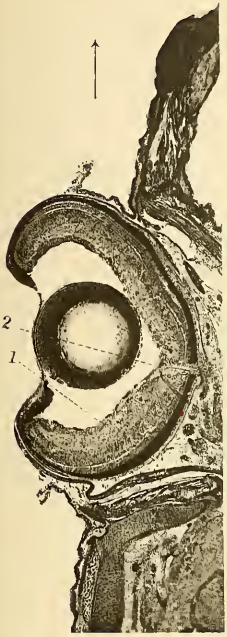

Fig. 59.

Fig. 60.

Fig. 61.

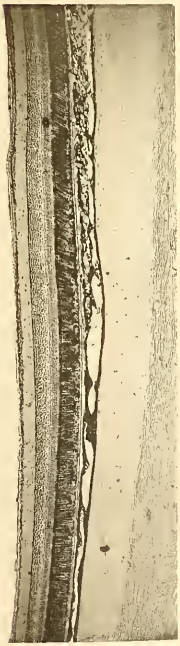

Fig. 62.

Fig. 63.

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EXPLANATION OF PLATE XXXI.

The following figures were made from sections through the whole head with eyes *in situ*. *FN* marks the axis of vision for the fovea nasalis; *FT*, fovea temporalis; and *Op*, the entrance of the optic nerve indicated by the pecten.

The slight divergence of the lines of binocular vision (*FT*) is probably due to the relaxation of the internal recti muscles after death.

FIG. 64. White-Bellied Swallow (Tachycineta bicolor), $\frac{3}{1}$.

FIG. 65. Common Tern (Sterna hirundo), $\frac{1}{1}$.

FIG. 66. Sparrow Hawk (Falco sparverius), $\frac{1}{1}$.

FIG. 67. Broad-Winged Hawk (Buteo latissimus), $\frac{1}{1}$.

FIG. 68. Great Horned Owl (Bubo virginianus), $\frac{1}{1}$.

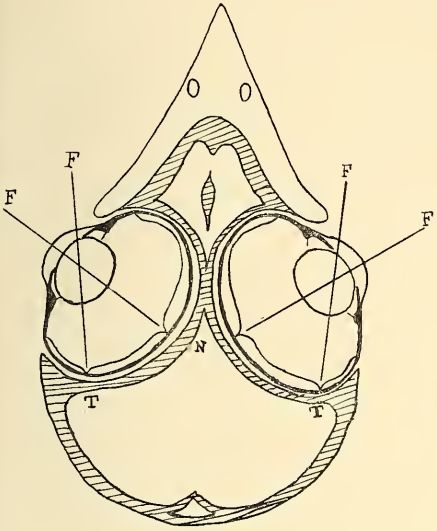

Fig. 64.

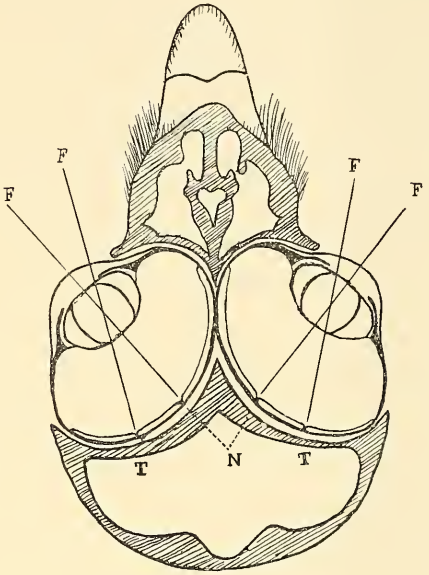

Fig. 67.

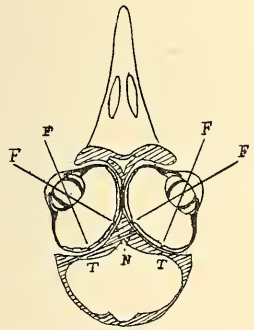

Fig. 65.

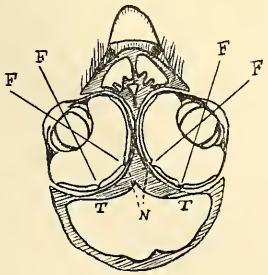

Fig. 66.

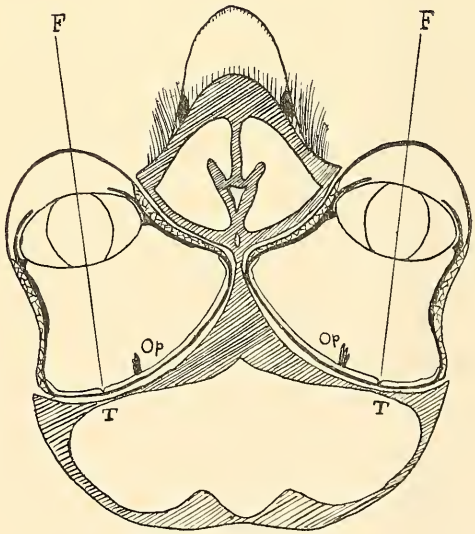

Fig. 68.

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